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Symposium on Primary Production: Preface

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Editor

Energy pathways in an ecosystem are tremendously complicated and involved, as indicated by the ordinary food-web diagram, making it difficult to think about production and productivity except with reference to a particular product. Only the first step in energy transformation is relatively simple. Here radiant energy from a single source—the sun—is fixed by photosynthetic plants as chemical energy in a variety of organic compounds. This constitutes the primary production of the ecosystem, and to the extent that allochthonous organic compounds and the activity of chemosynthetic autotrophs are at a minimum, the net "product" so formed supports the pyramid of heterotrophic organisms constituting the remainder of the biocoenosis.

During the early history of limnology and oceanography the standing crops of primary producers were laboriously studied by counting, weighing, or determining the volumes of the organisms. From surmises as to mean generation length, production coefficients, etc., estimates of the production accomplished were then attempted. These have had a limited usefulness because of the difficulty in collecting the necessary data and because of the lack of universal acceptability or applicability of the basic assumptions.

Within the past 20 years a number of methods have been developed for determining the actual rates of primary production. A summary of these and the results obtained thereby is of obvious value, and accordingly a symposium sponsored jointly by the American Society of Limnology and Oceanography and the Ecological Society of America was held on September 6, 1955, at East Lansing, Michigan. The participants and their topics are:

Ryther, J. H. Primary production and its measurement.

Riley, G. H. Primary production in the oceans.

Verduin, Jacob. Primary production in lakes.

Penfound, W. T. Primary production of vascular aquatic plants.

Odum, H. T. Primary production in flowing waters.

Ohle, Waldemar. Specific producitivity and its determination in waters.

The symposium was very well received by the large audience. Four of the papers presented appear on the following pages.

The Measurement of Primary Production[1]

JOHN H. RYTHER

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ABSTRACT

A discussion is presented of the measurement of primary production by short-term experimental methods and by measurement of *in situ* environmental changes occurring over longer periods of time. The methods described include the measurement of photosynthesis by oxygen production, carbon dioxide assimilation and the associated pH change, and uptake of radioactive carbon (C^{14}). *In situ* changes in the standing crop and the concentration of inorganic plant nutrients are also discussed, and the possibility explored of relating the rate of uptake of radioactive inorganic tracers to primary production. The relationship between chlorophyll and photosynthesis is reviewed and some new experimental evidence is presented which indicates the possibility of using chlorophyll concentration as an index of primary production. In the discussions of each method emphasis is placed on sensitivity, reliability, and the interpretation of the data.

The production of organic matter is carried out in water, as elsewhere, by plants through the familiar process of photosynthesis. The problem of measuring primary production in natural waters then resolves itself, with certain reservations, to the measurement of the photosynthetic rates of aquatic plant communities.

The present paper will be concerned with the methods by which production may be measured. Emphasis will be placed on theoretical considerations rather than the applied aspects of such measurements. The actual techniques which may be employed are governed to a large extent by the particular environment to be studied and the producer populations living therein, and various details of the problem require unique solutions.

The productivity of natural waters is often judged on the basis of the quantity of organic matter which they support at any one moment. This instantaneous population or "standing crop" in itself is a rather poor index of production without some indication of the time factor involved in its formation. Large beds of eel grass, kelp, drifting *Sargassum*, etc. may consist of organic matter which has been synthesized over a period of months if not years. The actual production rate of such impressive populations may be quite low and may be overshadowed by that of relatively small standing crops of phytoplankton which are subject to decimation from many sources. Production must therefore be considered as a rate rather than a static quantity.

The most direct and in many cases the most satisfactory method of measuring production is simply by the increase or decrease in the standing crop over a measured interval of time. In this case production is measured in terms of biomass, and may be expressed variously as the increase in, 1) the area of bottom covered, 2) volume of producer organisms, 3) displacement volume, 4) wet or damp weight, 5) dry weight, 6) ash-free dry weight (i.e., loss on ignition).

This method, in one form or another, has been widely used in studying the production of macroscopic benthic populations which follow a regular seasonal pattern of growth and are relatively undisturbed by predation or other decimating factors. It is quite unsuitable for measuring production of the phytoplankton since changes in its biomass reflect merely the net change resulting both from production and removal by predation, water movement, sinking, etc.

In the latter case it is desirable to measure as nearly as possible the instantaneous production rate. In practice this means

[1] Contribution No. 825 from the Woods Hole Oceanographic Institution. This work was carried out with the aid of a research grant from the National Science Foundation.

confining measurements to a few hours or, at most, days, rather than weeks or months. For this, one must turn to the reactions involved in organic production and the accompanying chemical changes which may be detected by more sensitive means. This leads to a brief consideration of photosynthesis.

In photosynthesis carbon dioxide is reduced to organic carbon with the evolution of free oxygen, the reaction utilizing the energy of solar radiation. Physiologists found early in their investigations of this process that the volume of oxygen liberated very nearly approximates the volume of CO_2 assimilated. These early workers reached the conclusion, still widely upheld, that the primary if not the sole product of photosynthesis is carbohydrate in nature. This then led to the familiar simplified equation for the overall reaction of photosynthesis:

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \qquad (1)$$

The subsequent discovery that the photosynthetic oxygen is derived not from CO_2 but from H_2O, necessitating a modification of the equation to satisfy the stoichiometry, need not concern us here since the combining proportions of the various constituents remain the same in either case.

While equation (1) is a convenient expression for the reaction of photosynthesis, it by no means describes the complex process of growth in which the first products of photosynthesis are converted to fats, proteins, organic acids, etc. with the accompanying uptake and utilization of the mineral nutrients. Growth, which is more analogous to "primary production" than photosynthesis alone, probably cannot be expressed by any simple equation. However, an examination of the composition of the organisms will give some insight into the combining proportions of some of the major constituents involved in this process.

Table 1 gives the per cent of ash-free dry weight (% organic matter) of carbon, hydrogen, oxygen, nitrogen, and phosphorus for a variety of aquatic plants. The list is by no means complete, but probably adequate to illustrate consistencies or major

TABLE 1. *Elementary composition of some aquatic plants in percent ash-free dry weight ± standard deviation*

Organism and Reference	No. Analyses	C	H	O	N	P
Diatoms	18	50.54	10.21	28.83	7.0	1.55
Brandt & Raben (1920)		±3.90	±1.90	±7.10	±3.7	±.88
Ketchum & Redfield (1949)						
Vinogradov* (1939)						
Peridineans	7	48.12	7.50	33.85	10.40	0.80
Brandt & Raben (1920)		±1.90	±1.70	±3.90	±1.40	±.36
Chlorophyceae	18	54.55	7.54	31.21	7.7	2.94
Ketchum & Redfield (1949)		±1.30	±.50	±4.35	±.56	±.83
Macroscopic algae	6	46.80	7.62	41.65	5.0	0.44
Brandt & Raben (1920)		±1.40	±1.90	±3.30	±2.40	±.37
Zostera	3	46.11	7.12	43.26	3.4	0.50
Brandt & Raben (1920)		±.60	±1.10	±1.70	±1.6	±.26

* N and P values only

variations in the relative composition of the five elements for the different groups represented. With this table for a guide, we may turn our attention to the applicability of the photosynthetic equation to the measurement of production. Before doing so, however, we may consider here briefly the effects of respiration upon such measurements.

The overall effect of respiration may be considered as the reverse reaction of photosynthesis. The organism exists in a state of equilibrium between these two processes, and changes in the standing crop or the related environmental factors reflect merely the product of this equilibrium.

Actually, ecologists are generally less concerned with the total photosynthesis or "gross production" than with the net effect of photosynthesis and respiration by the plant community, or "net production". The former is an intangible quantity; the latter represents the real production of organic matter which is added to the environment and hence is of ecological significance.

The rate of change of the standing crop, of course, measures "net production". Unfortunately the rate of change of the environment does not, for it reflects not only respiration of the producer population

but also that of all other forms of life in the community.

If production is measured experimentally for short periods of time, the effects of respiration may be eliminated by performing parallel experiments in the dark in which respiration alone is measured. This practice is valid only if respiration is the same both in the light and dark. This assumption is obviously not correct if any appreciable growth occurs during the experiment. Thus one's purpose is defeated by the very thing he is attempting to measure and at best the value obtained is approximate and somewhat below the correct value for production.

Even disregarding the problem of differential growth in the light and dark control, the question of whether the process of respiration itself is the same in the light and dark has always bothered students of photosynthesis. The most recent investigation of this problem would indicate that it is. Brown (1953) studied respiration in *Chlorella* whose oxygen supply was enriched with O^{17} (heavy oxygen). By following the time course of the disappearance of O^{17} from the air in equilibrium with the culture, he could measure respiration independently of photosynthesis, since the photosynthetically produced oxygen contained none of the heavy isotope. His experiments show that respiration is essentially independent of light.

OXYGEN PRODUCTION

According to equation (1) for each mole of CO_2 reduced to organic carbon one mole of free oxygen is liberated. As mentioned earlier, this observation antedated and actually provided the basis for the photosynthetic equation. We have emphasized that the same relationships may not hold for both photosynthesis and growth. But if environmental changes are measured under the natural growing conditions of the plants they should reflect the effects of both processes simultaneously.

Table 2 shows some of the measurements which have been made of the photosynthetic quotient, defined here as the ratio of oxygen produced to carbon dioxide assimilated. While the values shown do not differ markedly from unity, it is obvious that they tend to be somewhat greater. The general opinion is that these values do not differ significantly from one, though some of the authors (cf. Barker) consider that the slight departure indicates the synthesis of substances slightly more reduced than carbohydrates.

TABLE 2. *Some measurements of the photosynthetic quotient ($O_2/-CO_2$) in aquatic plants*

Organism	Reference	PQ
Chlorella	Manning *et al* (1938)	0.98
Chlorella	Sargent (1940)	ca. 1.00
Chlorella	Wassink and Kersten (1944)	1.09–1.12
Diatoms	Wassink and Kersten (1944)	1.12–1.13
Nitzschia closterium	Barker (1935a)	1.05–1.08
Nitzschia palea	Barker (1935a)	1.03–1.05
Peridinium sp.	Barker (1935b)	1.03–1.11
Synechoccus sp.	Fraenkel *et al* (1950)	1.08±0.03
Homidium flaccidum	van der Paauw (1932)	0.92–1.07
Gelidium	Tseng and Sweeney (1946)	0.94±0.05
Gigartina	Emerson and Green (1934)	0.99–1.07
Raw seawater	Sargent and Hindman (1943)	1.09±0.23

From general considerations, the proximity of these values to unity seems rather surprising, for the organisms are certainly not composed of carbohydrates alone. The synthesis of either fats or proteins would result in values considerably in excess of one, and it would seem doubtful that they could be balanced by the few organic acids more highly oxidized than carbohydrate. When one examines the composition of aquatic plants, the observed PQ values are still more difficult to explain. This is illustrated in Table 3 showing the relative composition by atoms of carbon, hydrogen, and oxygen in a variety of aquatic plants. They are obviously more highly reduced than carbohydrate and their PQ, calculated from their composition, ranges from 1.09 to 1.48, averaging about 1.20.

If the plants obtain their nitrogen in the form of ammonia, the PQ may be somewhat smaller since the nitrogen is already fully reduced when it enters the cell. If, on the other hand, nitrate is the principal source of nitrogen, as is normally the case in most natural waters, then the PQ will be still higher than that indicated in Table 3. While the mechanism is not clear, it is

TABLE 3. *The relative composition by atoms of C, H, and O (C = 100), and the calculated photosynthetic quotient (O_2/—CO_2) in various aquatic plants*

Reference and Organism	No. analyses	C–H–O	PQ
Brandt & Rabin (1920)			
Diatoms	8	100–251–48	1.38
Peridians	7	100–184–54	1.16
Zostera	3	100–179–70	1.09
Chorda filum	1	100–186–72	1.10
Laminaria	1	100–182–68	1.11
Ascophyllum	1	100–170–64	1.10
Ulva	1	100–170–65	1.10
Enteromorpha	1	100–196–51	1.23
Rhodomela	1	100–315–60	1.48
Ketchum & Redfield (1949)			
Stichococcus bacillaria	6	100–160–46	1.17
Chlorella pyrenoidosa	8	100–168–41	1.21
Chlorella vulgaris	1	100–168–44	1.20
Scenedesmus obliquus 1	1	100–180–41	1.25
Scenedesmus obliquus 2	1	100–180–39	1.25
Scenedesmus basilensis	1	100–184–39	1.27
Nitzschia closterium	1	100–164–42	1.20

TABLE 4. *Variations in the relative composition and calculated photosynthetic quotient (O_2/—CO_2) in* Chlorella pyrenoidosa (from Spoehr and Milner 1949)

Relative composition by atoms (C = 100) C–H–O–N	Calculated PQ nitrogen source		
	Not consid.	NH_4^+	NO_3^-
100–165–61–16	1.11	0.99	1.22
100–162–45–16	1.18		
100–171–39–23	1.23		
100–160–38–16	1.21		
100–173–43–8	1.21		
100–178–36–16	1.22		
100–168–37–12	1.26		
100–172–33–6	1.28		
100–168–28–4	1.31		
100–177–27–2.2	1.33		
100–179–20–1.3	1.34		
100–180–19–1.7	1.35	1.34	1.37

generally agreed that plants must reduce nitrate to ammonia before it may be used. The subject has been reviewed recently by Syrett (1954). Whatever the process, the effect upon the PQ is the same. This was nicely demonstrated by Cramer and Myers (1948). *Chlorella* grown under their experimental conditions had the composition $C_{5.7}H_{9.8}O_{3.2}N_{1.0}$. With ammonia as their nitrogen source the PQ calculated from the composition was 1.10 while that determined experimentally was 1.06. With nitrate-nitrogen the calculated and measured PQ were respectively 1.45 and 1.47.

In a subsequent paper, the same authors (Myers and Cramer 1948) provided an explanation for the fact that most earlier measurements of PQ have approached values of unity. If they made their measurements at the same light intensity at which the organisms were grown they obtained quotients of 1.3–1.4 (with nitrate as a nitrogen source). If, however, the organisms were grown at a low light intensity and measured at light saturation, values of about 1.10 were obtained. These workers concluded that when *Chlorella* is transferred from low to high light intensity, its metabolism is temporarily shifted to one involving principally carbohydrate synthesis. Myers strongly maintains that the concept of a PQ of unity has resulted from experiments with organisms grown at sub-saturating light and measured, because of the requirements of the manometric techniques, at high intensity.

The photosynthetic quotient, of course, varies with the composition of the organisms. Table 4, taken from Spoehr and Milner (1949) illustrates the variation in composition which may occur in the single organism, *Chlorella pyrenoidosa*, shown in increasing order of its state of reduction. The calculated PQ, disregarding the effect of nitrogen assimilation, ranges from 1.11 to 1.35.

Since synthesis of fats increases with nitrogen deficiency (cf. Spoehr and Milner) the more highly reduced the organism the lower is its nitrogen content. Hence the state of reduction of the cells and nitrate reduction do not together produce a much greater effect upon the PQ than either one may have separately, as illustrated in Table 4.

In view of the preceding evidence it would appear that a photosynthetic quotient of one is largely an experimental artifact, and that values of 1.1 to 1.3 or higher are normal under natural conditions. While it is obviously impossible to choose the cor-

rect value for routine measurements of natural populations, the author suggests that a value of 1.25 is perhaps somewhat more realistic than that of unity.

The photosynthetic production of oxygen is usually studied in the laboratory by means of elaborate manometric techniques which are quite unsuitable for use in the field, particularly if one wishes to measure the process as it occurs *in situ*. Ecologists have generally employed the technique first described by Gaarder and Gran (1927) of measuring the rate of change of dissolved oxygen titrametrically by the Winkler method in samples enclosed in glass bottles. Since these may be returned to the same depth, maintained at the same temperature and receive the same illumination as the surrounding water, an approximation may be obtained of the natural production rate.

The dissolved oxygen concentration is influenced in such experiments by the respiration not only of the plants but of all other life including the large bacterial flora which develops in water enclosed in bottles, so that the net change in oxygen is meaningless. One normally employs a darkened bottle, therefore, to correct for respiration and thereby measure gross production.

Such "light and dark" bottle experiments are generally comparable in sensitivity to the more elegant manometric techniques; each is capable of detecting changes of a few hundredths of a milliliter of oxygen per liter. But where the physiologist may confine his measurements to a few minutes by using extremely dense cultures of organisms, the ecologist must take his populations as he finds them and, in an extremely oligotrophic environment, may require a matter of days to detect a measurable increase in oxygen.

Experiments by the author have indicated that photosynthesis in samples enclosed in glass bottles may proceed at a constant rate for 24 to 48 hours after which it almost invariably decreases. As a general practice such experiments should probably not exceed the daylight portion of one day. The sensitivity of Winkler titrations is approximately 0.02 ml of oxygen per liter. Since two such titrations are involved in a "light and dark" bottle experiment, the author has considered a difference of 0.05 ml per liter as the lower limit of sensitivity of the method. This is equivalent to carbon assimilation of approximately 0.02 mg per liter. The daily gross production of most natural waters normally exceeds this limit and therefore permits measurements not exceeding the time limit suggested above.

Before leaving the subject of oxygen production, mention should be made of Steemann Nielsen's objection to the "light and dark" bottle experiments of long duration as used by Riley *et al.* (1949 and ref.) for determining production in the open ocean. Steemann Nielsen (1952) first proposed that a differential growth of bacteria in the light and dark bottles due to the lethal effects of sunlight resulted in the over-correction for respiration and a corresponding overestimation of photosynthesis. Subsequent experiments by Vaccaro and Ryther (1954) disclosed no difference between the growth or respiration of bacteria in light and darkened bottles in experiments lasting for several days.

More recently Steemann Nielsen (1954) has postulated as a basis for the same result (i.e., a difference between respiration of bacteria in the two bottles) the bactericidal effects of substances produced by the phytoplankton in the light bottles in conjunction with photosynthesis. In a later paper (Steemann Nielsen 1955) the same author claims to have demonstrated this effect.

It is noteworthy, however, that Steemann Nielsen's experiments do not provide any direct demonstration of this phenomenon. On the other hand those of Vaccaro and Ryther give direct contradictory evidence. In samples of a natural plankton population enclosed in darkened and clear bottles exposed to sunlight, there was no difference in the growth of bacteria during a period of six days despite the vigorous photosynthetic activity in the light bottle (Vaccaro and Ryther 1954, pg. 23, exp. II).

Steemann Nielsen's stated objective was to demonstrate that production values obtained by long-term "light and dark" bottle measurements are many times too high. While experiments of long duration

have many shortcomings, some of which have been discussed above, everything in the author's experience would indicate that values obtained by such experiments are too low.

CO_2 ASSIMILATION

The uptake of carbon dioxide is equivalent, mole for mole, to the production of organic carbon, and hence represents one of the most direct approaches to the measurement of primary production.

In all natural waters of alkaline pH, carbon dioxide exists in equilibrium with HCO_3^- and $CO_3^=$. Thus photosynthesis is not reflected by changes in concentration of CO_2 alone, but may be determined quantitatively only by following the level of the entire buffer system. Furthermore, there is evidence that at least some plants are capable of fixing bicarbonate ions directly. This subject has been reviewed by Steemann Nielsen (1947).

The only practical field method of measuring carbon assimilation from the entire buffer system is by pH change. The total CO_2 (that present in all forms in the buffer system) and its relation to pH may be determined for freshwater by the equations of Moore (1939) and for seawater by those of Buch, Grippenberg, Wattenberg, and Harvey, which have been conveniently summarized by Harvey (1945). This relationship breaks down in those areas, both freshwater and marine, in which photosynthetic activity is accompanied by the precipitation of calcium carbonate, and the method obviously cannot be used under such conditions.

Because of the buffering capacity of seawater and most freshwater, a relatively large uptake of CO_2 is necessary to produce a significant change in pH. With the equipment available in the average laboratory (i.e., a Beckman pH meter) a change of 0.1 pH unit probably represents the limit of sensitivity. This is equivalent to carbon assimilation between 0.1 and 0.5 mg/liter depending upon the alkalinity of the water. The lower limit would be typical of moderately soft freshwater, the upper limit representing seawater.

The daily photosynthetic rate of many freshwaters may exceed this lower limit and thus permit use of the method. But in lakes of high alkalinity and in the ocean a detectable pH change within 24 hours will occur only in such areas of high production as plankton blooms, vigorously-growing benthic populations, etc. Significant results may be obtained in more oligotrophic waters by artificially concentrating the organisms (cf. Verduin 1951) or extending the experimental period to more than one day. Either alternative represents a departure from natural conditions which should be avoided if possible. While pH changes of a few thousandths of a unit may be detected with fair accuracy by means of more sensitive potentiometers, it is doubtful if such instruments would prove reliable at their lower limit of sensitivity under field conditions.

THE FIXATION OF CARBON—14

The most recently developed experimental method of determining organic production is that of measuring the rate of uptake of radioactive carbon (C^{14}) by the plants. The method was first described by Steemann Nielsen (1952) and later reviewed by Ryther and Vaccaro (1954), and the technical details need not be repeated here. Although designed for measuring production of phytoplankton populations, it could easily be adapted for use with other aquatic plants.

Essentially the method consists of adding a small quantity of $C^{14}O_3^=$ to a sample of water containing its natural plankton population. After allowing photosynthesis to proceed for a suitable period, the plankton is filtered on filter paper capable of retaining all autotrophic organisms. The recently developed Millipore filter is particularly well suited for this purpose. The filter is then washed, dried, and its activity measured by standard counting techniques.

The assumption is made that the following relationship is true:

$$\frac{\text{Activity of phytoplankton}}{\text{Activity of } C^{14}O_3^= \text{ added}} (k) = \frac{\text{Total C assimilation}}{\text{Total C available}}$$

The constant (k) corrects for the isotope effect, since C^{14} is assimilated some 6% more slowly than C^{12} due to mass difference. The total available carbon may be calculated from the equations discussed earlier.

This method represents the most sensitive technique now in use for measuring photosynthesis under natural field conditions, and its sensitivity may be varied at will by simply adjusting the activity of the added C^{14}. For example, 10 μ-curies of C^{14} may be added to a 250 ml sample volume without increasing the carbon content by more than 0.03 mg/l, an insignificant amount even in soft freshwater. Assuming a 50% counting efficiency, this amount of C^{14} added to seawater will permit measurement of C^{14} uptake where photosynthesis is as low as 0.02 μg C/liter. In freshwater, with less available carbon, the sensitivity is correspondingly greater. There is probably no natural body of water in which photosynthesis does not exceed this limit within a few hours. The latest measurements of the author in the surface waters of the Sargasso Sea, a highly unproductive oceanic area, show a C^{14} uptake throughout the day equivalent to the assimilation of about 1.0 μg C/l.

In Steemann Nielsen's opinion the C^{14} method, as described above, measures the total or gross photosynthesis minus a small loss due to the respiration of the newly assimilated C^{14} during the course of the experiment. For a four-hour experiment, he placed this loss rather arbitrarily at 4%, and applied this correction to obtain values for gross production.

The present author (Ryther 1954) measured photosynthesis simultaneously by oxygen production and C^{14} assimilation in a pure culture of *Dunaliella euchlora*[2] which contained limiting quantities of nitrogen and phosphorus. In the early stages of growth, when cell division proceeded at an exponential rate, photosynthesis was about ten times as great as respiration. However, as a nutrient deficiency developed, photosynthesis decreased more rapidly than respiration until, under conditions of complete nutrient exhaustion and two weeks after growth had ceased, the P:R ratio was unity. In every case the uptake of C^{14} corresponded closely with the oxygen produced in excess of respiration. Use of a PQ of 1.25, as discussed earlier, would have made the agreement more striking. This led the author to conclude that C^{14} uptake provided a measurement of net production.

The objection has been raised (Steemann Nielsen, personal communication) that carbon dioxide rather than nitrogen and phosphorus was the limiting factor in these experiments, thus invalidating the results. This assumption was presumably the basis for his statement that these observations "seem obviously to be the result of unsatisfactory experimental techniques" (Steemann Nielsen 1955).

In this connection it may be pointed out that (1) pH in the experimental culture increased during the initial stages of rapid growth but thereafter decreased, ultimately reaching a value below that observed at the start of the experiment, and (2) addition of nitrogen and phosphorus to a duplicate culture two weeks after growth had ceased resulted, after a lag of 48 hours, in renewed exponential growth. Although the cultures were not continually agitated, it was felt that the use of an actively motile flagellate would prevent local deficiencies of CO_2 from developing immediately around the cells.

To further investigate the author's claim that C^{14} uptake measures net photosynthesis, a comparison was made of the two methods at different light intensities. Again a pure culture of *Dunaliella* was used, and gross and net oxygen production were compared with C^{14} assimilation.

Light intensity was measured incident to the experimental vessels (30 ml glass-stoppered bottles) with a calibrated Weston photoelectric cell. Graded light intensities were obtained with neutral density filters and illumination was provided by a bank of daylight fluorescent lamps. The light measurements were admittedly somewhat crude and the author does not pretend that the absolute values so obtained were exact.

[2] Originally described as *Chlamydomonas* sp. and correctly identified by Dr. Ralph Lewin.

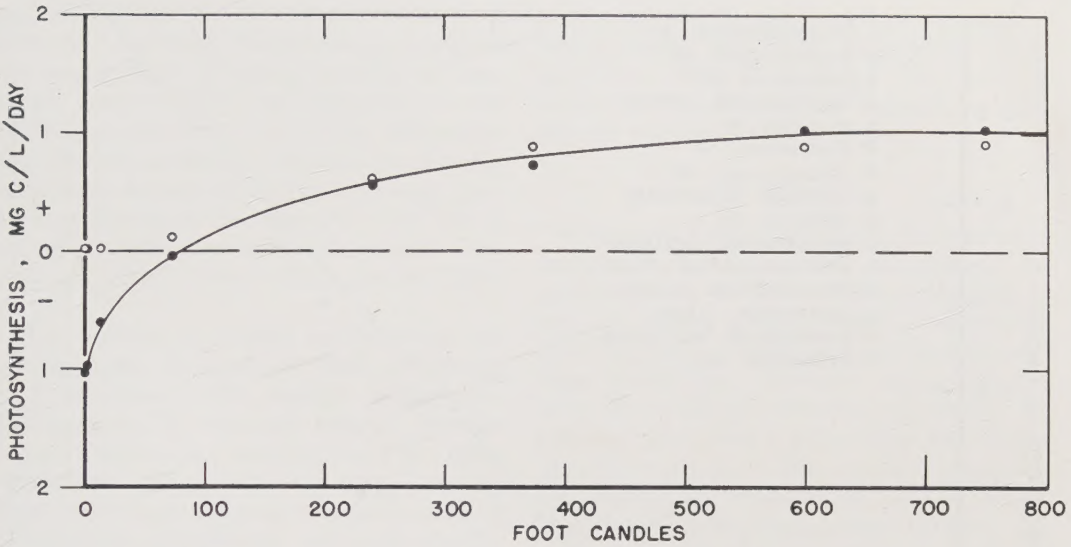

FIG. 1. Photosynthesis measured by oxygen production (filled circles) and C^{14} uptake (open circles) as a function of light intensity in a pure culture of *Dunaliella euchlora*. Values obtained from oxygen production represent the net change of oxygen at each light intensity.

For the purposes of this experiment it was considered sufficient merely to obtain a graded series of light intensities from saturation to values below compensation.

The results (Fig. 2) clearly show that C^{14} uptake agrees closely with the carbon equivalent of net oxygen production, in this case using a PQ of 1.25. Below the compensation intensity, where there was measurable photosynthesis but no net increase of oxygen, the uptake of C^{14} did not differ significantly from that which occurred in total darkness.

The reason that C^{14} uptake should correspond to net photosynthesis is not clear. It was originally suggested (Ryther 1954) that the plants may respire the newly formed products of their photosynthesis, and thus a portion of the assimilated C^{14} would be respired in the course of the experiment in the same ratio as normal P:R in the organism. However, it is rather difficult to believe that a complete equilibrium of this sort could be attained almost instantaneously, as would be required. Furthermore this hypothesis is not supported by the evidence of others (i.e., Calvin 1949, Weigl *et al.* 1951).

An alternative and perhaps more logical explanation is that the plants use respiratory CO_2 preferentially to external CO_2 for photosynthesis. In this case, the organisms would never assimilate external CO_2 in excess of their net photosynthesis. Some evidence for this is given in Warburg's (1920) experiments on the narcotization of photosynthesis by cyanide in which he found that only net photosynthesis was affected by the poison, the organism continuing to photosynthesize at a rate corresponding to its respiration. Supplementing this evidence Kok (1951) has recently found that the quantum yield of photosynthesis appears to be twice as great below the compensation point as that at light intensities above compensation. Both of these pieces of evidence appear to substantiate Franck's (1949) hypothesis that plants are capable of the photochemical reduction of respiratory intermediates. While Franck proposed that this occurs only below compensation light intensity, one need only extend his hypothesis to include the preferential use of these intermediates at all times to explain the situation reported above.

Whatever the correct physiological explanation, the empirical evidence seems clear that C^{14} uptake measures net photosynthesis. Upon first examination (including that of the present author) the

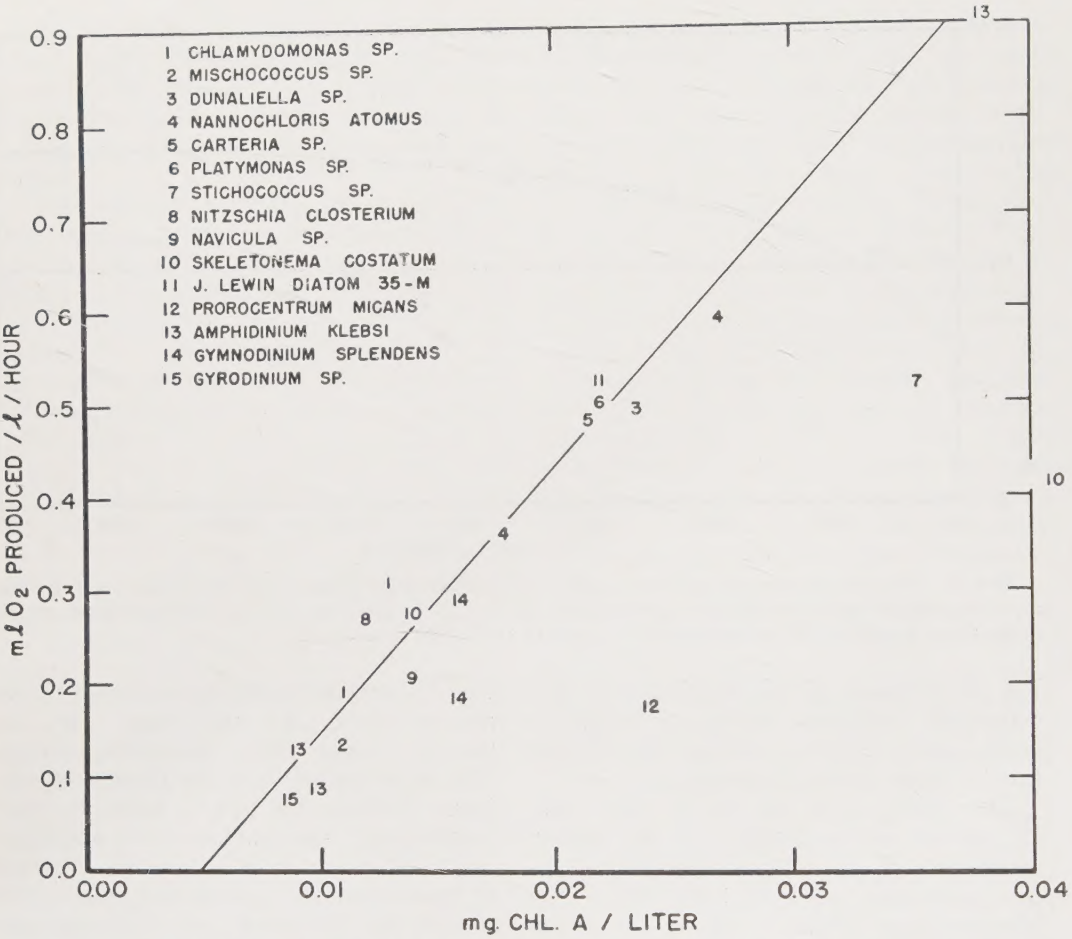

FIG. 2. The relationship between total photosynthesis at constant light intensity and chlorophyll "a" in some marine plankton algae.

method would appear to fulfill a long awaited need in providing the means for the direct measurement of net productivity. However, the fact must not be overlooked that net photosynthesis is thereby measured only during the course of the experiment (i.e., during the hours of the day when there is net photosynthesis to measure). The investigator would like to obtain values for overall net production on a daily basis. This information cannot be had from the uptake of C^{14}, for there is no way of estimating the additional respiratory loss of organic matter during the hours of darkness.

This fact greatly restricts the usefulness of the C^{14} method. While it remains a valuable tool for laboratory investigations and may provide interesting information pertaining to the relative fertility of natural waters, it does not provide, as currently employed, a quantitative measurement of either gross or net daily production.

The need is therefore apparent for a continued critical examination of the method and its physiological basis, and for the exploration of means to circumvent its shortcomings.

THE UPTAKE OF MINERAL NUTRIENTS

The uptake of nitrogen, phosphorus, and other mineral nutrients from the environment has been used in the past to estimate primary production. Since the changes involved are generally small, the method does not lend itself readily to experimental

measurements by ordinary chemical methods. In areas where there is a marked seasonal growth of plants, usually in temperate waters where the regeneration and buildup of nutrients during the unproductive winter months is followed by a rapid utilization during the spring flowering, production has been calculated *in situ* over a period of weeks or months (Kreps and Verjbinskaya 1930, Atkins 1923, Cooper 1938, etc.).

The method is subject to the errors resulting from changes of these properties not associated with organic production. Furthermore, it does not account for the regeneration and reutilization of these substances during the growing season. Finally the ratio between the concentration of nitrogen, phosphorus, and undoubtedly the other mineral nutrients and the total organic matter is extremely variable (Tables 1 and 4).

In general, estimates of production based on such criteria have represented the attempted extension of investigations in which the chemical cycles of the water have been the primary object of interest. Values which have been obtained in this manner are given by Riley (1941) and Harvey (1945).

The short term uptake of many of the mineral nutrients may be measured experimentally by means of radioactive tracers. For instance, P^{32} is available with a specific activity of 1 μ-curie per 0.025 μg P. The addition of 10 μ-curies (0.008 μgA P) per liter would not significantly increase the phosphorus content of any natural waters. The uptake of less than 10^{-5} of the total available phosphate could then be detected by normal counting equipment. This is probably exceeded by the daily phosphorus assimilation of the poorest of producer populations.

The difficulty arises in trying to relate P uptake to organic production. The reader is again referred to Table 1 as an illustration of the inconsistency of the phosphorus content of aquatic plants, not only between various groups but also within the groups as indicated by the standard deviations. Several workers have shown that a large proportion of the assimilated phosphorus is loosely bound and freely exchanged (Goldberg *et al.* 1951, Rice 1953). This fraction cannot be quantitatively related to the organic content of the organism.

CHLOROPHYLL

Riley *et al.* (1949 and ref.) and others have attempted to relate the chlorophyll of aquatic plants to their total organic matter and, by following the change in the chlorophyll content of natural waters, thereby estimate production. However, many workers have shown that the chlorophyll content per cell may vary over a wide range depending upon the state of nutrition of the organisms, the quality and intensity of light in which they have grown, and other factors.

A much closer relationship appears to exist between chlorophyll and photosynthesis at a given light intensity. This fact was first demonstrated by plant physiologists who attempted to determine the smallest quantity of chlorophyll capable of reducing one mole of CO_2 to organic carbon (Emerson 1929, Arnold and Kohn 1934, Emerson and Arnold 1932, Gaffron and Wohl 1936). Fleischer (1935) showed that the photosynthesis:chlorophyll ratio remained constant even in cells whose chlorophyll content varied widely as a result of deficiencies of iron and nitrogen, though the relationship did not hold for magnesium deficient cells.

In a series of nine Wisconsin lakes, Manning and Juday (1941) found at the depth of maximum photosynthesis that 1.0 mg of chlorophyll related to the mean production of 6.7 mg of oxygen per hour with a standard deviation of only 0.60.

Edmondson (1955) observed a close correlation ($r = 0.86$) between photosynthesis per unit light and chlorophyll in a group of cement tanks at Woods Hole which contained phytoplankton populations resulting from the enrichment of natural seawater. This relationship was largely independent of changes in the species composition of the tanks.

Many plants develop more chlorophyll when grown in the shade than when exposed to bright light, and Sargent (1940)

TABLE 5. *Photosynthesis by* Dunaliella euchlora *grown at 350 foot candles (shade cultures) and 1500 foot candles (sun cultures) at different light intensities*

Intensity (foot candles)	Photosynthesis: mg carbon/4 hrs/10^8 cells		Photosynthesis: mg carbon/4 hrs/mg Chl. "a"	
	(shade cult.)	(sun cult.)	(shade cult.)	(sun cult.)
7000	0.34	0.06	2.30	1.80
4700	0.49	0.17	3.20	5.00
2400	1.30	0.28	8.70	8.00
950	1.50	0.42	10.00	12.00
280	0.73	0.17	4.80	4.80
126	0.36	0.07	2.40	2.10

observed this effect in *Chlorella*. He found, however, that his "sun cultures", though containing less chlorophyll per cell, carried on photosynthesis at any light intensity at a higher rate than the "shade cultures". In other words, photosynthesis per unit of chlorophyll appeared to vary according to the light intensity at which the organism was grown.

This was not the case in some recent experiments carried out at this laboratory. Duplicate cultures of *Dunaliella euchlora* were grown at light intensities of approximately 350 and 1500 foot candles under fluorescent lamps. After two weeks growth their chlorophyll "a" content was 1.5 mg and 0.35 mg per 10^8 cells respectively.

These cultures were diluted and C^{14} added to aliquots which were then lowered to various depths in Woods Hole Harbor. Photosynthesis was then measured over a four-hour, mid-day period, incident radiation was measured with a recording photoelectric cell calibrated in foot candles, and the extinction coefficient of visible light measured with a submarine photometer. The results of this experiment (Table 5) illustrate that, although the amount of chlorophyll per cell and the rate of photosynthesis per cell are extremely variable, the rate of photosynthesis per unit of chlorophyll remains relatively constant at any given light intensity regardless of the intensity at which the organisms grew.

Some preliminary experiments by the author and G. C. Fraser with 15 species of phytoplankton, including green algae, diatoms, and dinoflagellates, indicate a constant relationship between chlorophyll "a" and photosynthesis at a constant light intensity. Chlorophyll "a", determined from the equations of Richards with Thompson (1952), was considered rather than total chlorophyll since the accessory pigments (chlorophylls "b" and "c") vary widely among different taxonomic groups and do not participate directly in photosynthesis. Photosynthesis was determined by the difference in oxygen concentration between darkened and clear bottles exposed to a bank of fluorescent lamps for six hours. The experiments were made with stock cultures of the organisms without regard to their cell concentration, age, or physiological condition.

There was a considerable reduction and scatter in the photosynthesis:chlorophyll ratio in the denser cultures which is believed to have resulted from the reduction of light by the organisms themselves. At concentrations below 0.04 mg chlorophyll "a" per liter there was a rather good linear relationship observed (Fig. 2). Since neither light intensity nor cell concentrations were measured in these experiments, they lack any useful quantitative significance, but they are nevertheless valuable for demonstrating the constancy of the photosynthesis-chlorophyll "a" relationship among a wide variety of marine plankton algae.

The task remains, then, of obtaining precise quantitative measurements of chlorophyll "a" and photosynthesis at different light intensities for a few representative species of plankton algae. With that information, the measurement of a few variables such as daily surface radiation, the extinction coefficient of visible light, and chlorophyll "a" concentrations at various depths within the euphotic zone would provide adequate data for an estimate of total daily production within an entire body of water.

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Primary Production in Lakes

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ABSTRACT

The mean photosynthetic rate of lake phytoplankton under optimal light (natural conditions) is about 0.5 micromoles of O_2 evolved or CO_2 consumed per microliter of organisms per hour; 1–2 micromoles per milligram ash-free dry weight per hour; and 0.2 micromoles per microgram of chlorophyll per hour. Simple equations are presented for computing photosynthetic yield per square meter of water surface. Computations for several lakes yielded values mostly lying between 150–200 millimoles per square meter per day. A year-round study under completely natural conditions in western Lake Erie showed winter yields of about 40 millimoles per square meter per day and summer maxima of about 300. The annual curve followed the solar radiation curve closely. It is estimated that 28 days are required for a gram of phytoplankton (ash-free dry weight) to produce an additional gram of glucose available for reproduction; 80 per cent of the photosynthate produced is required to support phytoplankton respiration.

Three quantities, related to primary production in lakes, are measured by aquatic biologists: (1) the volume of autotrophic organisms, (2) the ash-free dry weight of suspended particles (organic seston), and (3) the concentration of chlorophyll—each of these expressed per unit volume of water. These are measures of standing crop, and they represent roughly quantitative estimates of the population of autotrophic organisms upon which primary production is based.

Some biologists have studied photosynthesis under approximately natural conditions and have related the observed O_2-production or CO_2-consumption to one or more of the quantities named above. From such data it is possible to compute mean photosynthetic yields per unit of standing crop. Table 1 presents a summary of such yields computed from the literature. These data represent total or true photosynthesis, i.e. the experiments included darkened bottles to take account of the CO_2 contributed by respiration, or of the O_2 so consumed. Riley's (1941) data are included because they permit computation in all three categories, and because they show that the rates for marine phytoplankton are in general agreement with those for fresh water phytoplankton. The data in this table suggest that the mean photosynthetic rate of a microliter of autotrophic organisms, under optimal light (natural conditions) amounts to about 0.5 micromoles of O_2 evolved or CO_2 consumed per hour (6 μg C). The rate per milligram of ash-free dry weight is about 1 or 2, (12–24 μg C) and the rate per microgram of chlorophyll is about 0.2 micromoles of O_2 (2.4 μg C). These values represent numerous tests and can be regarded as fairly reliable means, but results from individual tests vary widely. The data represent epilimnion collections. Manning and Juday (1941) state that hypolimnion samples exhibit yields which are less than one-third so high.

A survey of the literature shows that there is a rough inverse correlation between the depth of the epilimnion (or total depth in unstratified lakes) and the standing crop of autotrophic plants (Manning and Juday 1941, Pennak 1949, Verduin 1954). Thus lakes with an epilimnion layer of the order of one meter are likely to have standing crops of the order of 50 microliters of plant volume per liter of water, 15 milligrams ash-free dry weight, and 30 micrograms of chlorophyll. Lakes having an epilimnion depth of the order of 10 meters are likely to have standing crops about one-tenth so high. These values are intended to indicate order of magnitude only. Large variations in standing crop are observed, of course,

TABLE 1. *Phytoplankton photosynthesis under optimal light*

To indicate variation observed routinely among individual samples write $\times \div 3$ after each value in the table. To convert to micrograms CO_2 multiply by 44, O_2 by 32, carbohydrate by 30, carbon by 12.

Micromoles CO_2 absorbed or O_2 evolved per			Reference
μlit/hr	mg/dry-wt/hr	μg chlorophyll/hr	
	0.8	0.22	Manning & Juday (1941)
		0.22	Gessner (1949)
0.5	1.6		Verduin* (1956)
	2.0		Jackson & McFadden (1954)
0.6	1.3	0.31	Riley* (1941)

* Average of year-round research under natural conditions. The other data represent summer conditions. All are in temperate zone.

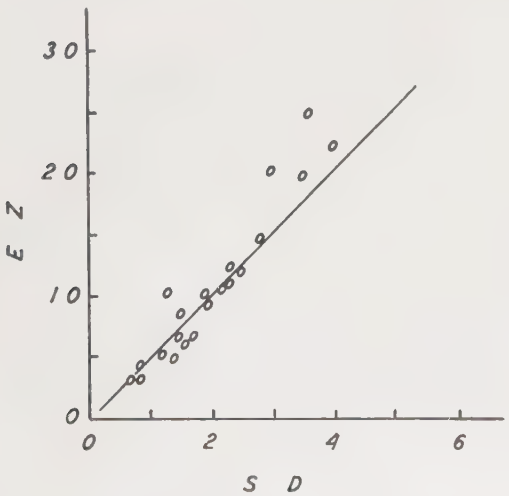

FIG. 1. Relation of Secchi disc reading (SD, meters) to depth associated with one per cent of surface light (EZ, meters). An approximate estimate of euphotic zone is obtained by multiplying Secchi disc reading by 5.

from time-to-time in a given lake and among different lakes of similar epilimnion depth.

The data in Table 1 can be used to provide estimates of photosynthesis in a given lake if the following quantities are known: (1) the standing crop, expressed as plant volume, organic seston, or chlorophyll, (2) the depth associated with one per cent of surface light, which marks the lower limit of the euphotic zone (Ruttner 1953: 127), and (3) the ratio of mean photosynthetic rate in the euphotic zone to the rate under optimal light. Estimates of standing crop are abundant in the literature. Light penetration data are less abundant, but if Secchi disc readings are included a considerable amount of information is available. To make Secchi disc readings approximately convertible to euphotic zone depths, simultaneous determinations with the Secchi disc and submarine photometer were made during August 1955 by the crews of two vessels assigned to the synoptic survey of Lake Erie. The data are plotted in Figure 1. The line, drawn by inspection through the scatter diagram, suggests that an approximate estimate of the depth of euphotic zone can be obtained by multiplying Secchi disc readings by 5. The scatter in Figure 1 indicates that the relationship is not precise. Different conversion factors appear in the literature; Riley (1941) used a factor of 3; from the data of Bursche (1955) a factor of about 2.5 can be computed; Rawson (1950) suggests that the factor decreases as transparency increases, and he lists a factor of 4.3 when the Secchi disc reading is about 1 meter.

The ratio of mean photosynthesis in the euphotic zone to the rate under optimal light is about 0.65. This value has been determined from numerous experiments in western Lake Erie (Verduin 1956) and can be derived from Figure 12 of Manning and Juday (1941).

The following equations are now presented for computing approximate daily photosynthesis per square meter of water (Y_a).

$$Y_a = P_v \times Y_p \times D_1 \times 0.65 \qquad (1)$$

Where P_v is mean phytoplankton crop per cubic meter in the euphotic zone, Y_p is daily photosynthesis per unit standing crop under optimal light (Table 1), and D_1 is the depth of the euphotic zone. This equation applies to lakes whose euphotic zone is confined to the epilimnion, or to unstratified lakes showing 1 per cent or less of surface light on the bottom.

Because photosynthetic yields in the hypolimnion are so much smaller than in the epilimnion (Manning and Juday 1941) a

modified equation is presented for lakes whose euphotic zone extends a considerable distance into the hypolimnion, or for unstratified lakes showing light intensities considerably in excess of 1 per cent of surface light on the bottom:

$$Y_a = P'_v \times Y_p \times D_2 \qquad (2)$$

Where P'_v is mean phytoplankton crop in the epilimnion, Y_p is as above, and D_2 is depth to midthermocline, or to bottom in unstratified lakes. In this equation it is assumed that the entire standing crop in the epilimnion is exposed to optimal light, and that the contribution of the hypolimnion is negligible. The first assumption is overoptimistic, and the second is unduly pessimistic. They, therefore, tend to counteract each other, yielding a simple equation which is likely to provide a fair approximation of the photosynthesis yield.

To test the usefulness of these equations they have been used to compute Y_a in seven Wisconsin lakes studied by Manning and Juday (1941). The yields from equations (1) and (2) are compared in Table 2 with yields computed by Manning and Juday, using a detailed method (estimating light intensity and photosynthetic yield at 1-meter intervals in the vertical column, and at hourly intervals throughout the day) which was admittedly more refined than the data warranted. In applying equations (1) and (2) I used the standing crop of chlorophyll determined by Manning and Juday, and the hourly photosynthetic yield reported by them (Table 1, of this paper), and assumed that there were 12 hours of daylight during which the photosynthetic rate was maintained. The roughest approximation in this type computation is the failure to correct for dim light when the sun is low, or obscured by clouds. Steemann Nielsen has shown (1954: 317) that marine photosynthesis (per m^2) is not linearly proportional to light intensity, but that an intensity corresponding to one-third of full sunlight will support a photosynthetic yield amounting to two-thirds of the rate at full sunlight. And I have shown (Verduin 1956) that photosynthetic yields on cloudy days were considerably higher than one would predict on the basis of observed light intensities. The approximation, therefore, is not so rough as would at first appear, and Table 2 shows close agreement, in most cases, between the yields computed from equation (1) or (2) and those reported by Manning and Juday. It seems likely that these simple equations provide as reliable an estimate of the photosynthetic yield per square meter of water as does the more tedious method outlined by Manning and Juday.

TABLE 2. *Photosynthesis per square meter per day (Y_a) computed from equation (1) or (2) as appropriate, compared with yields computed by Manning and Juday (1941)*

Lake	Yield by Manning and Juday (mmol)	Yield from equation (1) or (2) (mmol)
Helmet	47	89 (1)
Trout	53	56 (1)
Crystal	57	58 (2)
Muskellunge	57	72 (1)
Weber	83	78 (2)
Nebish	87	96 (2)
Scaffold	146	325 (2)

It should be emphasized that the least reliable factor in these equations is Y_p, the photosynthetic rate per unit of standing crop. I believe that significant differences can be established between rates for different lakes. Manning and Juday did not study this aspect of the problem. The low value of photosynthetic rate (per mg ash-free dry weight) for Wisconsin lakes (Table 1) as compared to Lake Erie, Pymatuning Reservoir, and Georges Bank, is evidence that such differences exist.

Pennak's publication (1949) provides the data needed for yield computations using equation (1) or (2). In Table 3 these yield estimates are presented. Pennak's standing crops are expressed as organic seston, a photosynthetic rate of 1.4 micromoles per mg per hour was used to compute the yields, and Secchi disc readings were multiplied by 5 to provide euphotic zone depths. The actual determination of Y_p and of euphotic zone depths in Colorado lakes might result in a considerable revision of the values in Table 3. Computations for western Lake Erie, Sandusky Bay, and some roadside ditches in Kansas are included. The most

TABLE 3. *Photosynthesis per square meter per day computed from organic seston and light penetration data for some Colorado lakes, and others*

All yields were computed using a value of 1.4 millimoles per gram ash-free dry weight per hour for the PS yield per unit of phytoplankton crop, and assuming a 12-hour day. Where D_1 is given equation (1) was used, and where D_2 is given equation (2) was used.

Lake	Organic Seston (grams/m²)	Euphotic zone depth (meters, D_2)	Depth to mid-thermocline or bottom (meters, D_1)	Photosynthesis (mmol)
Allens	1.9	7		145
Base Line	1.7		6	170
Haydens	1.8		5	152
Beasley	2.4		4	162
Gaynor	13.6	3		450
Boulder	4.7	5		255
Kossler	1.2		6	122
(Pennak 1949)				
Erie	2.8	5		153
(Verduin unpubl.)				
Sandusky	13	1.4		200
Bay (McQuate unpubl.)				
Kansas	15		0.6	151
ditches (Ratzlaff 1952)				

FIG. 2. Photosynthesis (PS, mmol/m²/day) under completely natural conditions in western Lake Erie, graphed against time of year. The yield is proportional to the solar radiation curve (broken line, hundreds of g.cal/cm²/day).

interesting feature in Table 3 is the uniformity of yield estimates. Most lakes show yields between 150 and 200 millimoles (1.8 and 2.4 grams C) per square meter per day. Gaynor Lake, in Colorado, shows a considerably higher yield; consequently that would be a most interesting one in which to determine actual photosynthetic yields per unit of organic seston and actual depths of euphotic zone.

In Figure 2 the monthly mean photosynthetic yields per square meter per day for waters in the island region of western Lake Erie are graphed against time of year. These estimates are based on observed pH changes in the bay near the F. T. Stone Laboratory between early morning and late afternoon (Verduin 1956), and they represent photosynthetic rates measured under completely natural conditions. A line has been drawn through the highest values observed at various seasons of the year. The values lying below this line (February and April) were associated with high turbidities which severely limited photosynthesis. The clear waters which are usually encountered a few miles to the northwest, or east, of the island region (Verduin 1954) almost certainly supported yields similar to those observed when the currents were accomodating enough to move clear water into the bay.

The curve in Figure 2 bears a striking similarity to the curve for radiant energy flux in this latitude (Verber 1955) which has been drawn in as a broken line. It is significant that maximal yields were observed in June, a month which has been consistently associated with minimal diatom crops (Chandler 1944, Chandler and Weeks 1945, Verduin 1951).

Under completely natural conditions CO_2 consumption during daylight hours is very nearly equal to CO_2 evolution during darkness (Verduin 1956). Therefore the ratio of photosynthesis by the autotrophic community to respiration by the total community must be close to unity. It is difficult to distinguish between the respiratory contribution of autotrophic organisms and that of the other members of the community. In vigorous laboratory cultures algal respiration usually represents about 10 per cent of maximal photosynthesis (Ryther 1954), and when diatom populations from Lake Erie, concentrated using No. 25 bolting cloth, were studied (Verduin 1956) the mean ratio of respiration to maximal photosynthesis was 0.125. If this value is regarded as representative of the autotrophic community an estimate of

phytoplankton reproduction can be made for western Lake Erie as follows: Photosynthesis under optimal light is 1.6 millimoles per gram ash-free dry weight per hour (Table 1), or

$$\left(\frac{1.6 \times 0.65 \times 12}{2}\right) = 6.2 \text{ mmol/g/day.}$$

The factor of 2 is introduced because the euphotic zone represents about one-half of the total water mass (Verduin 1954). Respiration of the autotrophic community amounts to about $(1.6 \times 0.125 \times 24) = 5.0$ mmol/g/day. The photosynthate available for reproduction amounts to about 1.2 mmol/g/day, or approximately 20 per cent of the total photosynthetic yield. This estimate is similar to Riley's (1941) Table VI, from which a mean total plankton production representing 15 per cent of total gross production can be computed. Converting to glucose one obtains $(1.2 \times 30) = 36$ mg/g/day. Thus it would require $\frac{1}{(0.036)} = 28$ days for a gram of ash-free dry weight to produce an additional gram of glucose available for reproduction. This estimate of regeneration time agrees in general with Einsele's turnover coefficient (0.83 per month) and Grimm's observation of annual diatom yield (8–10 times maximal standing crop) as reported by Ruttner (1953: 149–151).

DISCUSSION

Of the three quantities used as a basis for computing photosynthetic yield (Table 1), organic seston would seem to be the least reliable estimate of autotrophic organisms because important fractions of non-autotrophic organisms and of detritus are included. The variation among individual samples, however, is high regardless of the base used, and is only slightly higher in the organic seston-based rates than in the others. (See Riley's 1941 correlations between these bases and oxygen production, for example.) It is noteworthy that the average rates computed from Riley's work are about 50 per cent higher than those from the work of Manning and Juday (1941) in both the ash-free dry weight- and the chlorophyll-based columns. The difference, therefore, does not reflect a higher fraction of non-autotrophic material in the Wisconsin organic seston. If the curve in Figure 2 is used to compute an average photosynthetic yield (per m^2/day) a value of about 140 mmol is obtained. This agrees closely with the value of 153, for Lake Erie, in Table 3. The latter is based on centrifuged organic seston determinations and an assumed photosynthetic yield of 1.4 mmol/g/hr. This comparison is particularly significant because Figure 2 represents CO_2 change under completely natural conditions and is not based on estimates of phytoplankton volume, organic seston, or chlorophyll. These observations suggest that organic seston values (means from numerous determinations) provide a fairly reliable index of photosynthetic capacity in spite of the large variations in ratio of autotrophic to non-autotrophic material which must be present among individual samples. However, a method which would provide even a roughly quantitative distinction between autotrophic organisms, non-autotrophic organisms, and detritus would contribute much to aquatic biology

It should be emphasized that the production estimates in this paper represent rough approximations. They should provide a motive (not a substitute) for more detailed research in this field. The large variation in photosynthetic yield among individual samples (Ryther 1954, Verduin 1956) makes the study of numerous samples mandatory regardless of the precision achieved in individual determinations. Consequently, a time-consuming method which provides a high degree of precision may be less valuable than a more rapid method with a larger scatter which will disappear when many samples are averaged. Moreover, any detailed study of one aspect must be accompanied by equally detailed determination of other aspects. For example, careful determinations of photosynthetic rates will not improve the area-based yield estimates if determinations of light penetration are not equally refined. And the actual euphotic zone depth for a specific community is a function of that community's efficiency under dim light; the roughly approximate

"1 per cent of surface light" must be adjusted to fit specific communities and climates. When such refinements are attempted the choice of tool used for light intensity measurement becomes important. I have pointed out (Verduin 1956) that photronic cells like those in the photometers used at the Stone Laboratory are relatively insensitive to non-visible radiation, and that the photosynthetic process also is insensitive to non-visible radiation. For primary production studies, therefore, this type of photometer has advantages over the pyrheliometer type, because the latter measures the non-visible rays which represent about one-half of surface radiation but are ineffective in photosynthesis and are rapidly screened out by water.

This difference in instruments is responsible for Rawson's (1950) conclusion that the ratio between euphotic zone depth and Secchi disc reading decreases with increasing transparency. In a private communication Dr. Rawson sent me the original graph on which his regression equation was based. This graph included data from western Lake Erie in which a photronic cell was used and data from Wisconsin lakes in which a pyrheliometer was used. The Wisconsin surface values, therefore, contained the non-visible fraction while the Lake Erie surface values excluded it. Thus a systematic error was introduced by graphing these different types of data on a single graph. The error was aggravated by the fact that most of the low transparency data came from Lake Erie and the higher transparency data from Wisconsin. The regression equation determined by Rawson, therefore, is unreliable, as can be verified by examining the extremes: at zero depth of euphotic zone the predicted Secchi disc reading is 0.3 meters, and beyond 14 meter depth of euphotic zone the predicted Secchi disc reading exceeds the depth of euphotic zone. Although this analysis clarifies in part the discrepancy in published ratios between Secchi disc readings and euphotic zone depth, the problem warrants more detailed study because the Secchi disc is used widely and its value can be enhanced by a better understanding of the variables involved.

In a previous publication (Verduin 1956) I demonstrated that less than half of the photosynthesis observed under natural conditions can be accounted for by the phytoplankton crops detected by examination in the Sedgwick-Rafter cell. Figure 2 further emphasizes that fact. Lake Erie, like many temperate zone lakes, exhibits vernal and autumnal diatom maxima with winter and summer minima. The annual curve for photosynthesis under natural conditions is not bimodal, but shows a summer maximum at a time when the diatom crops are at a minimum. It seems likely that other lakes which exhibit bimodal phytoplankton abundance curves will reveal similar unimodal productivity curves. The organisms responsible for this unexplained photosynthetic activity (their identity, size, biomass, metabolic activity, etc.) present one of the major problems in primary production today.

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Primary Production of Vascular Aquatic Plants

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ABSTRACT

In this paper, data are presented on the productivity of four hay crops, five prairie plots, one floodplain community, one cultivated aquatic crop, two emergent plant populations, and two floating mat communities. The productivity (production rate) of the communities investigated varied with the amount of light, water, and nutrients available. The average productivity, in grams of carbon per square meter per day, based on the terminal crop, was moderate (1.5) in hay crops, tall grass prairie and rice, relatively high (3.6) in giant ragweed and presumably still higher in certain aquatic plants. The terminal standing crop was found to be less than the sum of periodic measurements of the developing crop. It was noted also that the magnitude of productivity values depended upon the time of harvest. The productivity of vascular aquatic plants was usually highest in spring and autumn and lowest during the summer. Low summer productivity was due primarily to the relatively low rate of photosynthesis, compared with that of respiration, during hot summer weather. On the basis of present data it appears that productivity in the terrestrial habitat was greatest along shorelines of water bodies and did not increase continuously in the hydrarch succession toward the regional climax.

INTRODUCTION

The problem of the relative productivity of terrestrial and aquatic communities has been of continuing interest to limnologists and oceanographers. Odum (1953) believed that land, fresh-water, and marine habitats might be equally productive and that productivity depended upon the quantity of energy from the sun, the amount of raw materials, limiting factors, and the plants and animals present. In this paper, data are presented on the productivity of four terrestrial hay crops, five tall grass prairie plots, one floodplain community, one cultivated aquatic crop, two emergent plant populations, and two floating mat communities. In comparing the production and production rates of these populations it is hoped that a better insight may be gained into the relative productivity of terrestrial and aquatic habitats.

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TYPES OF VASCULAR HYDROPHYTES

The organisms within an ecosystem may be grouped into a series of trophic levels in which the producers, or green plants, are directly dependent upon solar radiation as a source of energy (Lindeman 1942). Of the producers, the vascular aquatic plants (vascular hydrophytes, rooted hydrophytes, or macrophytes) are characteristic of relatively shallow areas in the littoral zone. In addition to rooted plants, the vascular hydrophytes include unattached, free-floating plants which have roots (*Azolla*, *Lemna*, *Piaropus*, etc.) and others which lack roots (*Wolffia* and *Wolffiella*). Vascular aquatic plants include several species of pteridophytes, and an impressive number of species of monocotyledons and dicotyledons. In several investigations the stoneworts (Characeae) and certain mosses (*Drepanocladus* and *Fontinalis*) have been included in the standing crops of vascular aquatic plants.

A vascular aquatic plant is one that grows in soils usually covered with water during a major portion of the growing sea-

son (Hess and Hall 1945). Vascular aquatic plants include both woody and herbaceous representatives, and occur in both salt water and fresh water habitats. In the United States, there are relatively few woody plants that grow in salt water. Among these is the well-known red mangrove,[1] *Rhizophora mangle*, which grows in water to about four feet in depth and is associated with several submerged herbaceous halophytes in deeper water. Aquatic trees are much more common in fresh water, the most widespread being baldcypress, *Taxodium distichum*, and tupelo gum, *Nyssa aquatica*.

The herbaceous vascular hydrophytes are usually divided into emergent, floating, and submerged species. Zonation apparently occurs, however, in each of these groups. Most emergent species occur in water less than one foot deep but some species (e.g. *Dianthera americana*) may thrive in water six feet deep. The floating hydrophytes have been divided into pleuston, floating mat, and floating leaf plants by Hess and Hall (1945). Species of smaller floating leaf aquatics (*Alisma*, *Heteranthera*, *Hydrocotyle*, *Monniera*) occur typically in water less than two feet deep, whereas the larger, and possibly more important, floating leaf aquatics (*Brasenia*, *Castalia*, *Nelumbo*, *Nymphaea*) occur in relatively deep water. In submerged plants there is considerable zonation. Apparently there are three belts (in progressively deeper water) which might be characterized as angiosperm, stonewort, and moss zones. Among the submerged species the stoneworts (Characeae) are usually thought to occur in the deepest water. However, Rickett (1924) found *Drepanocladus pseudo-fluitans* in the deepest water in Green Lake, Wisconsin, and Hasler (1938: 95) discovered two mosses, *Fontinalis* and *Drepanocladus*, "at the astonishing depth of 394 feet (120 meters)" in Crater Lake, Oregon.

LIMNOLOGICAL ROLE OF VASCULAR HYDROPHYTES

Vascular aquatic plants are of considerable importance to small aquatic animals since they provide support, shelter, food, and oxygen. When decay occurs vascular hydrophytes "contribute directly to the stock of organic detritus which is so important an element of subsistence for insects and even for some fish" (Coker 1954: 200). Stauffer (1937) found that when the eel grass, *Zostera marina*, around Woods Hole was wiped out in 1931, nearly all the animals once found living on or among the eel grass disappeared with it. This demonstrates that vascular hydrophytes may be absolutely necessary for the survival of many aquatic animals. The superb summary of Hotchkiss (1941: 160) on the limnological role of higher aquatic plants indicates, however, that they may be a mixed blessing for aquatic animals. "Higher plants make lakes more habitable for waterfowl and fishes, but . . . help to destroy the habitat for both themselves and their animal associates. They add oxygen . . . but cut down on the ability of the water to absorb it. . . . They furnish ducks with essential food, but their contribution of decomposing material may periodically help to reduce oxygen to the point where botulism can develop and take its toll. They support an abundance of fish food, but their dense growths may favor an increase of snails and other intermediate host of fish parasites."

The productivity of animals in an ecosystem varies with the type of aquatic plant and its influence on the physical factors of the environment. Emergent plants favor high productivity of migratory waterfowl and aquatic mammals. Submerged species, on the other hand are especially useful in the production of animals which spend all, or most of, their lives in the water. Floating plants vary greatly in their capacity to support aquatic animals. Pleustonic plants, such as *Azolla caroliniana*, *Lemna minor* and *Spirodela polyrhizza*, are known to reduce the oxygen content of water bodies far below the minimal requirements of fish. Floating leaf plants such as *Brasenia purpurea*, *Nelumbo lutea* and *Nymphaea odorata*, also reduce the oxygen tension of bodies of water although not so drastically as the pleustonic species.

Plants that form floating mats produce

[1] All plant names are according to Small's *Flora of the Southeastern United States*, 1913.

anaerobic conditions that are very unfavorable to animals living in a water medium. In a floating marsh of papyrus (*Cyperus papyrus*) in Africa, Beadle (1952) reported that the watery layer below the floating mat was free of oxygen except near the outer lake edge and that even patches of open water were devoid of oxygen except for the surface film. Similar conditions have been noted in mats of water hyacinth (*Piaropus crassipes*) and alligatorweed (*Achyranthes philoxeroides*) by Lynch *et al.* (1947). They stated that, "The water hyacinth blankets fresh water ponds and stream, destroying the fish therein, and ruins the waterfowl habitat. Alligatorweed is an even more serious threat to wildlife and fisheries, since it thrives on dry land and in fresh and brackish marshes." The annual damage caused by these floating leaf aquatics has been estimated at five million dollars for the state of Louisiana, but the yearly loss in all the southeastern states is probably three to four times this amount. In view of the destructiveness of these species one is forced to conclude that one is measuring 'zoological destructivity' and not biological productivity when measuring production rates in these species.

GENERAL PRINCIPLES OF PRIMARY PRODUCTIVITY

Dice (1952: 150) states that, "The ultimate limit of productivity of a given ecosystem is governed by the total effective solar energy falling annually on the area, by the efficiency with which the plants in the ecosystem are able to transform this energy into organic components, and by those physical factors of the environment which affect the rate of photosynthesis." According to Coker (1954: 17), "Productivity, as far as it depends on solar radiation, is a function of surface area rather than of volume of water." Lindeman (1942) found that the total productivity in shallow Cedar Bog Lake, Minnesota, per cubic meter of water was very high, but that the actual yield per square meter was less than one-third that of Lake Mendota, Wisconsin. He attributes this to the fact that less light is available for photosynthesis by phytoplankton and vascular hydrophytes in the shallower water body. Another explanation is possible, however. The total nutrients may be much greater in a relatively deep lake than in a shallow lake. In a very shallow lake there is a relatively small nutrient volume for phytoplankton; rooted aquatics have a limited supply of nutrients and may, at times, have an inadequate supply of water, especially in the prairie and plains states.

Lindeman (1942: 415) believed that productivity declines "with lake senescence, rising again in the terrestrial stages in hydrarch succession" and that "productivity tends to increase until the system approaches maturity." This may be true for hydrarch successions in cold-temperate regions but does not appear to be the case in southern United States. In general the cottonwood-willow forest type occurs along the borders of streams, ponds, and lakes. "Of all the bottomland hardwoods, cottonwood grows the fastest and yields the greatest volumes per acre per year" (Bond and Bull 1946). In Oklahoma, growth is fastest in the cottonwood-willow forest, slower in the elm-ash-hackberry bottomland forest, and least in the so-called regional climax—the blackjack-post oak forest. Our experience indicates that productivity, in terrestrial communities, may be greatest "at the water's edge" and does not increase continuously in the hydrarch succession toward the regional climax.

According to Welch (1935) the greater the development of the higher aquatic vegetation the greater the biological productivity of a body of water. However, he pointed out that certain lakes, with abundant rooted vegetation, were very low in plankton, whereas other lakes with few rooted hydrophytes, had an unexpectedly large plankton crop. He concluded, therefore, that the higher aquatic vegetation, as an index of productivity, should be used with caution. Prescott (1939: 72) stated "there is overwhelming evidence that open water lakes which support a rich macro-flora also maintain high phytoplankton productivity. . . ." Hasler and Jones (1949) found, however, that dense growth of large aquatic plants,

in small experimental silo-ponds, had a statistically inhibiting effect upon phyto- and rotifer plankton. In a recent study Moore (1952) reported that Lake Chicot, Louisiana, was heavily grown up with rooted aquatics, especially *Ceratophyllum demersum* and *Cabomba caroliniana*, and that phytoplankton productivity was very low. He suggested (p. 45) "that the higher aquatic vegetation . . . was effectively utilizing a large proportion of the available nutritive materials, thereby limiting the production of plankton." At Lake Providence, Louisiana, Moore found that there were few vascular aquatic plants. He ascribed this to the restricted amount of shoal area, "and to the abundant phytoplankton which not only limited light penetration but also was a considerable drain on the available supply of nutrients" (Moore 1950: 87). Apparently the relative abundance of macrophytes and phytoplankton in a given water body is largely a matter of competition—especially for light and nutrients. After reviewing the problem Welch (1952: 309) concluded, as he had in 1935, that "as an index character of general biological productivity the larger aquatic vegetation must be employed, for the present at least, with caution. . . ." It is known that a number of vascular terrestrial plants produce substances which inhibit the growth of other plants. In view of this fact, it is possible that such inhibiting substances may prove to be factors in the relative abundance of vascular hydrophytes and phytoplankton.

In this discussion the term productivity is synonymous with the rate of production (MacFadyen 1948). It represents the quantity of plant material produced per unit of time and may be designated by Q/T where Q denotes quantity and T equals time. Yields are given in terms of dry weight of living plant material, in pounds per acre or grams per square meter. Except for grain and hay crops, which are usually reported as air-dry weight, all data are presented in terms of oven-dry weight. For facility of comparison all production rates are reported as grams of oven-dry weight per square meter per day or as grams of carbon per square meter per day ($gC/m^2/day$), which in this paper will be referred to as GCMD. This discussion is based primarily on the GCMD values. The usual methods of arriving at this datum (GCMD) are: (1) total annual crop divided by the number of days for its development, and (2) periodic measurements of a developing crop.

PRODUCTIVITY IN TERRESTRIAL PLANTS

Most of the data available for terrestrial crops are based on the total annual crop divided by the number of days necessary for maturation. In hay crops only about three-fourths of the aerial parts are removed from the field. On the other hand all data on hay crops are based on air-dry weight (5 to 15 per cent moisture), a weight factor which helps to compensate for the stubble material left in the field. The productivity of several hay crops (U.S.D.A. 1954) has been calculated and is presented in Table 1. Perhaps the most significant finding is that all hay crops, except wild hay, have about the same production rate, from 1.47 to 1.76 grams of carbon per square meter per day. A GCMD value of about 1.5 seems to be representative for the average hay crop. Although alfalfa has the greatest annual yield of any crop listed it has about the same GCMD value as other hay crops because of its longer growing season (Table 1).

Productivity data on native prairie hay are available from the University of Oklahoma Grassland Plots near Norman, Oklahoma. Kelting (1954) found that the yield of living material was significantly greater on a moderately grazed pasture than it was on a decadent virgin prairie (Table 2). The GCMD value in the grazed pasture (1.75)

TABLE 1. *Average yield and productivity of common hay crops in the United States, 1943–1952*

Data from crop production annual summary for 1954.

Hay Crops	lbs/acre	g/m²	Days	g/m²/day	
				Dry wt.	Carbon
Alfalfa	4420	495	150	3.30	1.47
Clover-timothy	2820	316	80	3.95	1.76
Green grain	2400	269	75	3.59	1.60
Wild	1700	191	120	1.59	0.71

TABLE 2. *Primary productivity, in oven-dry weight of living material, of a tall grass prairie near Norman, Oklahoma*

Grazing data from Kelting 1950; plowing and mulching data from Rice and Penfound 1951.

Treatments	lbs/acre	g/m²	Days	g/m²/day	
				Dry wt.	Carbon
Influence of grazing					
Virgin prairie	2872	322	*105	3.06	1.37
Grazed prairie	3684	413	105	3.93	1.75
Influence of plowing and mulching					
Control (unplowed)	3060	343	†120	2.86	1.28
Plowed prairie	4639	520	120	4.33	1.93
Plowed-mulched	5656	634	120	5.28	2.36

* Harvested July 26; †harvested August 10.

was similar to that for average hay crops (1.5) but much higher than that for average wild hay (0.71) (Tables 1, 2). Rice and Penfound (1954) discovered that plowing augmented the productivity of native prairie and that mulching the plowed prairie increased it still further (Table 2). The GCMD value of 1.28 in the control (native prairie) was similar to that of average grain and hay crops (1.5) but much lower than that of the plowed (1.93), and plowed-mulched (2.36) plots. They found, also, that the oven-dry weight of all roots and rhizomes to a depth of seven inches was similar to the weight of the tops. This suggests that the total productivity (roots, rhizomes, and shoots) in the control, the plowed, and the plowed-mulched prairie plots should be much higher than the values given in Table 2.

In the tall grass prairie, many of the prevernal and vernal species had disappeared by midsummer, and considerable death of plant parts (especially basal leaves) occurred in the remaining species. Furthermore, the period of greatest biomass was attained in the prairie about August 1, before full flowering and fruiting had been accomplished. These facts suggest that the terminal standing crop in the prairie is not as great as the total yield of the community during the growing season.

Data on production rates are available on the giant ragweed, *Ambrosia trifida*, for the growing season of 1949. This large terrestrial weed was growing in a nearly pure stand along a tributary creek of the South Canadian River near Norman, Oklahoma. The number of plants, per square meter, decreased from 1840 to 110 during the growing season. By the end of the growing season an average of 70 per cent of the leaves on each plant had died and fallen off. These facts suggest that the terminal standing crop is somewhat less than the total yield of the ragweed stand during the growing season. The oven-dry weight, per unit area, increased continuously except for the final period (Table 3). The GCMD values were high in April-May, were moderate during the summer, were high again in the August-September period and showed a decrease during the fruiting period (Table 3). This productivity distribution parallels the pattern observed in phytoplankton, which is often characterized by spring and autumn pulses and low summer production. It will be observed that the GCMD values were higher than those of average hay crops (1.5) during the entire growing season (except the terminal period). The GCMD value of 3.57 for this particular stand of giant ragweed, based on the entire growing season, was also much higher than that of the average hay crop (Tables 1, 3). This high productivity was due, undoubtedly, to the rich, moist, floodplain soil in which this vigorous weed was growing.

TABLE 3. *Primary productivity of giant ragweed in the summer of 1949, Norman, Oklahoma*

Periods	End of period		g/m²/day	
	Plants/m²	g/m²	Dry wt.	Carbon
Apr. 23	1840	160		
Apr. 23–May 21	1220	580	15.0	6.70
May 21–June 15	740	690	4.4	1.96
June 15–July 21	370	900	5.8	2.59
July 21–Aug. 20	280	1090	6.3	2.81
Aug. 20–Sept. 17	110	1570	15.5	6.92
Sept. 17–Oct. 13	110	1550	−0.8	−0.36
Apr. 23–Sept. 17	110	1570	9.6	4.29
Apr. 23–Oct. 13	110	1550	8.0	3.57

PRODUCTIVITY OF VASCULAR AQUATIC PLANTS

According to Wilson (1939: 118), "The number of published studies that have been made with reference to the weight of the total crop of rooted hydrophytes are very few and entirely inadequate for any reliable correlations with the density of the fish population, or with other factors." Apparently the above statement is as true today as when published.

Rice is the only aquatic crop plant for which productivity data have been procured (U.S.D.A. 1954). The average annual grain yield of rice (2172 pounds per acre) was found to be much higher than that of other common grain crops as reported in the crop production summary for 1954 (corn, 1999; oats, 1066; wheat, 1020). In the case of rice it was found that the grain weighed slightly less than the straw (Masefield 1949). It would appear, therefore, that an appropriate, but conservative, estimate of total top yield might be obtained by doubling the weight of the grain. When this was done the average calculated GCMD value for rice was 1.55 (Table 4). This value (1.55) is similar to that of the average hay crops (1.5). It should be pointed out, however, that the relatively low GCMD value in rice is due to the long growing season (140 days) as compared to that of most hay crop plants (Table 1).

Data on an emergent hydrophyte, lizard's tail (*Saururus cernuus*), were provided by Dr. R. W. Kelting, University of Tulsa, in the summer of 1955. He determined the oven-dry weights of 30 plants in the medium fruiting stage. The calculated GCMD value was 2.45, a value somewhat higher than that of average hay crops but considerably lower than that of the giant ragweed (3.57).

Periodic measurements of a developing crop of the broadleaf cattail, *Typha latifolia*, were made at Norman, Oklahoma, in the spring and early summer of 1955. Although growth was well under way, all aerial parts were killed to the soil surface by a very destructive freeze on March 25. Growth was evident again by March 29, was relatively slow during April, very rapid during May but comparatively slow in June and July, when flowering and fruiting occurred.

The productivity was much greater than was expected. Even during the relatively cold weather of April the GCMD values were two to three times those of common hay crops (Tables 1, 5; Fig. 1). During May the productivity was prodigious, being many times that of average hay crops in the United States. Beginning with the hot weather of July the GCMD values dropped rapidly, with a considerable loss being registered by July 16 (Table 5; Fig. 1). This loss was due to continuing death of individual plants, to death of lower leaves (not included in weights), to insect depredation, and probably also to the relatively low rate

TABLE 4. *Average production and productivity of rice in the United States, 1943–1952*

Data from crop production annual summary for 1954.

Region	Grain lbs/acre	Grain g/m²/day	*Grain and straw g/m²/day	
			Dry wt.	Carbon
Louisiana	1806	1.45	2.90	1.29
Arkansas	2157	1.73	3.46	1.54
Texas	2126	1.70	3.40	1.52
California	3102	2.49	4.98	2.22
United States	2172	1.74	3.48	1.55

* Grain multiplied by two.

TABLE 5. *Productivity in broadleaf cattail,* Typha latifolia, *in the spring-summer of 1955, Norman, Oklahoma*

Periods	End of period	g/m²/day		
	g/m²	Dry wt.	Carbon	
			Period	‡Cumulative
Mar. 29–Apr. 15	147	8.6	3.84	3.84
Apr. 15–May 4	344	10.4	4.64	4.51
May 4–May 28	1080	52.6	23.48	10.04
May 28–June 7	1422	34.2	15.27	10.94
June 7–June 18	*1440	1.6	0.71	9.33
June 18–July 2	1527	6.2	2.77	8.17
July 2–July 16	†1245	−20.0	−8.93	5.71
July 16–July 30	†1051	−13.9	−6.21	4.15

* Cold, flooded part of period; †very hot, many leaves dead; ‡standing crop divided by days from start of growing season.

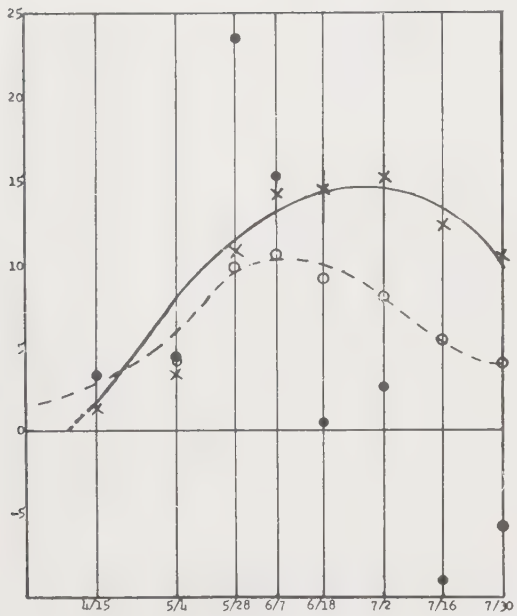

FIG. 1. Standing crops (hectograms of oven-dry weight per square meter) and productivity (grams of carbon per square meter per day) in the broadleaf cattail. ×, standing crop; ●, productivity per period; ○, cumulative productivity.

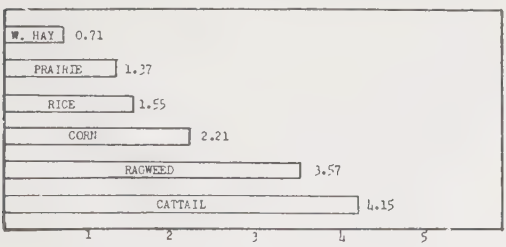

FIG. 2. Productivity of terrestrial and vascular aquatic plants in grams of carbon per square meter per day. All based on terminal standing crop except cattail.

of photosynthesis compared with that of respiration.

The largest standing crop of broadleaf cattail was not attained until July 2 (Fig. 1). One might expect that the highest seasonal productivity would occur at about the same time. Examination of the data, however, shows that the cumulative GCMD values were highest on June 7 and declined continuously thereafter (Table 5). These data indicate that the values relevant to seasonal productivity depend upon the time of harvest. It should be emphasized that there

TABLE 6. *Productivity of water cabbage,* Pistia stratiotes, *at Silver Springs, Florida*

Data from Odum 1954

Dates	g/m²/day	
	Total	Carbon
Aug. 18–Oct. 9	8.9	4.0
Oct. 9–Nov. 15	−8.4	−3.8
Nov. 15–Feb. 12	−1.8	−0.8
Feb. 12–Mar. 7	2.0	0.9
Mar. 7–Apr. 9	2.7	1.2
Apr. 9–May 11	13.0	5.8
May 11–May 23	15.3	6.8
May 23–June 9	6.5	2.9
June 9–July 2	3.1	1.4

was considerable turnover in the stand of cattail studied, with many plants dying and new ones being produced from the rhizomes, and that the terminal standing crop was less than the total productivity during the growing season. The above data suggest, also, that productivity data based on the terminal standing crop are not comparable with those based on periodic measurements of the developing crop (Figs. 1, 2).

The cage method has been utilized by Odum (1954) for estimating primary productivity. In the water cabbage (*Pistia*) Odum found that the highest production rates occurred in April-May with a secondary peak in August-October (Table 6). It will be observed that the GCMD values in water cabbage were much higher in April-May, and again in August-October, than in hay crops or in rice. It should be pointed out, however, that both the aerial and underwater parts were used in water cabbage, whereas only the aerial parts were harvested in crop plants. Production rates in the water hyacinth followed the same general pattern as in water cabbage—rapid production in early spring, low productivity during the summer, accelerated growth in the autumn, followed by a loss in living plant material during the winter months (Penfound and Earle 1948). The relatively low summer productivity may be due to the fact that the maximum rate of photosynthesis occurs at much lower temperatures than the maximum rate of respiration. As a matter of fact, the rate of photosynthesis is

often less than the respiratory rate during periods of hot days and nights in midsummer (Transeau *et al.* 1953: 163). It is probable, also, that the accelerated productivity in the spring and again in the autumn is related to the comparative rates of photosynthesis and respiration.

Odum (1954) ascertained that the production rate in *Sagittaria* at Silver Springs, Florida, in pounds per acre per year, was only 5,800 from November 15 to February 12, whereas it was 62,700 from June 9 to July 2. The calculated GCMD values were 0.79 and 8.60 for the respective periods, the latter (8.60) being much higher than that of *Pistia* at any period of the year. The GCMD values for *Sagittaria* and *Pistia* indicate that productivity in vascular aquatic plants varies not only from season to season but also from species to species.

Data on productivity of water hyacinth have been provided through the courtesy of Dr. T. T. Earle, Tulane University. He tagged six plants and weighed them, and their vegetative offshoots, every two weeks for a period of two months during the summer of 1955. He found that the total wet weight of the plants increased continuously from June 23 to August 6. Under similar conditions, it had been found that there were 32 water hyacinth plants per square meter and that the oven-dry weight was five per cent of the total wet weight (Penfound and Earle 1948: 458, 462).

By combining the above facts with the data supplied by Dr. Earle, the calculations in Table 7 were obtained. With this procedure it should be recognized that the calculated values are only approximate.

TABLE 7. *Productivity of water hyacinth in 1955 at New Orleans, Louisiana*
Data from Dr. T. T. Earle, Tulane University.

Dates	g/m² at end of period		g/m²/day	
	Wet wt.	Oven-dry wt.	Dry wt.	Carbon
Start on June 23	8800	440	—	—
June 23–July 9	12864	643	12.7	5.7
July 9–July 23	16760	838	13.9	6.2
July 23–Aug. 6	20864	1043	14.6	6.5
Aug. 6–Aug. 23	25515	1276	13.7	6.1

However, the range of these values (5.7 to 6.5) was very slight and was within the amplitude expected. The cumulative GCMD value for the two month period was 6.1. The GCMD values were much greater than those of hay crops, tall grass prairie, and rice (Tables 1, 2, 4) and were somewhat higher than those calculated for water cabbage (Tables 6, 7). It should be emphasized, however, that these values are minimal, since the number of water hyacinth plants per square meter (32) was very much less than were present in crowded stands.

PRIMARY PRODUCTIVITY IN DIVERSE HABITATS

The relative primary production in aquatic and terrestrial habitats has been of much interest to limnologists and oceanographers. Rickett (1922, 1924) reported oven-dry weights of 1801 and 1590 pounds per acre for submerged vascular plants in Lake Mendota and Green Lake, Wisconsin, respectively. Natelson (1955) reported standing crops of 4686, 3667, and 3774 pounds per acre of rooted vascular plants in three spring-river systems in Florida. In Wisconsin the standing crops of submerged vascular hydrophytes were somewhat lower than average hay crops (Table 1) and unplowed tall grass prairie (Table 2) and much lower than the crops of submerged hydrophytes in Florida. It should be pointed out, however, that the vascular plants, both in Wisconsin and Florida, constituted only a part of the total standing crop. At Silver Springs, Florida, *Sagittaria* accounted for only 30 per cent of the total primary production (Odum 1954). These facts suggest that the total primary production (submerged hydrophytes and phytoplankton) in Wisconsin may be similar to that on land and that primary production in the spring-river systems in Florida may be much greater than in terrestrial habitats.

Allee *et al.* (1949) compared the productivity of a lake, a tall grass prairie, and a deciduous forest. They believed that natural tall grass prairie might have a higher production rate than average field corn if allowance was made for stratification and a longer growing season. They felt that they were justified, therefore, in increasing "the

daily yield by 30 per cent over the average corn figure" (p. 507). If the roots (7.3 per cent of crop) are omitted, the calculated GCMD value for Transeau's "100-bushel corn" is 6.20 and that of average corn (35.7 bu/acre) is 2.21. However, the highest GCMD values for unplowed native prairie in Oklahoma ranged from 1.28 to 1.75 (Table 2). In view of the evidence, it appears that the productivity of native prairie should be much lower than that postulated by Allee *et al.* (1949).

In discussing crop plants and forest trees, Transeau *et al.* (1953: 184) concluded that "in all kinds of crops the average annual accumulation of energy per acre is about 6 million Calories. In an acre of forest it is about 10 million Calories." On the basis of the above figures the deciduous forest should have a total annual production of about 1.67 times that of crop plants. Since the productivity of hay crops and tall grass prairie is similar (Tables 1, 2) the annual yield in a deciduous forest should be about 1.67 times that of a tall grass prairie. Given a representative yield in native prairie of about 3,000 pounds per acre, the annual yield in a forest would be 5,010 pounds per acre. If we assume growing seasons of 120 days for native prairie and 180 days for deciduous forest, GCMD values of 1.25 for tall grass prairie and 1.39 for deciduous forest are obtained (Table 8). These data suggest that the daily yield of native, tall grass prairie is similar to that of a deciduous forest. It is probable, also, that bumper crops, such as the "100-bushel corn" of Transeau (1926), considerably exceed the productivity of a deciduous forest. It will be observed that the calculated GCMD values for tall grass prairie and deciduous forest from Allee *et al.* (1949) are much too high when compared with the adjusted values (Table 8) and those obtained by other investigators on the tall grass prairie (Table 2).

TABLE 8. *Estimated yield of glucose and oven-dry weight in three types of communities*

Data in first column from Allee *et al.* 1949.

Community	Glucose lbs/acre/day	Glucose g/m²/day	Carbon, g/m²/day	
			Calculated	Adjusted
Lake	54	6.05	2.42	2.42
Tall grass prairie	100	11.21	4.48	1.25
Deciduous forest	125	14.01	5.60	1.39

Whether a lake, a tall grass prairie, or a deciduous forest has the greater production rates is still unknown. In all three habitats, light, water, and nutrients are important environmental factors. In a lake, light, nutrients, and sometimes oxygen, are usually the limiting factors in productivity. Undoubtedly water and nutrients are paramount factors in the daily yield of prairie plants. Although one might expect greater productivity in a forest, because of extensive stratification, it is well to remember that water and nutrients are often the limiting factors in forest productivity, especially on the deciduous forest frontier.

SUMMARY

The productivity of vascular aquatic plants has been investigated. These plants are known to be of considerable value to aquatic animals since they provide support, shelter, food, and oxygen. Floating mat plants, such as alligator weed and water hyacinth, however, have been shown to be very destructive of aquatic animal life.

The productivity (production rate) of both vascular terrestrial and vascular aquatic plants was found to vary greatly with the amount of light, water, and nutrients available.

The average productivity, in grams of carbon per square meter per day, based on terminal standing crops, was moderate (1.5) in hay crops, tall grass prairie, and rice, relatively high in giant ragweed (3.6) and presumably still higher in certain aquatic plants.

The terminal standing crop in vascular plants has been found to be less than the sum of periodic measurements of the developing crop. Since the magnitude of productivity values depends upon the time of harvest it is obvious that great care should be exercised when comparing these values.

The productivity of vascular plants was often high in spring and autumn and rela-

tively low during the summer. This low summer productivity in vascular plants was due primarily to the relatively low rate of photosynthesis, compared with that of respiration, during hot summer days.

On the basis of present data it appears that productivity in the terrestrial habitat is greatest along shorelines of water bodies and does not increase continuously in the hydrarch succession toward the regional climax.

The question of the relative productivity of a lake, tall grass prairie, and deciduous forest has been discussed. Although no final conclusion was reached, it was found that the productivity values for tall grass prairie and deciduous forest, as quoted in the literature, were much too high.

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Primary Production in Flowing Waters[1]

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ABSTRACT

Respiration, photosynthetic production, and diffusion interact to produce the daily curve of oxygen change in a segment of flowing water. Conversely, the observed curves of oxygen in streams can be used to calculate the component rates of production, respiration, and diffusion. New production values obtained with these analyses of oxygen curves from various sources, as well as a few previously existing estimates of primary production, indicate a generally higher rate of production in flowing waters than in other types of aquatic environments.

The ratio of total primary production to total community respiration is used to classify communities quantitatively according to their predominantly heterotrophic or autotrophic characteristics. Longitudinal succession within a stream tends to modify the ratio towards unity from higher values for autotrophic and from lower values for heterotrophic communities. The behavior of this ratio is described for the annual cycle in a stream, for the sequence of pollution recovery, and for diverse types of communities.

INTRODUCTION

To the casual eye the biota of flowing waters is rich. Coral reefs, river rapids, tidal channels, and the lush plant beds of calcareous streams seem to be full of life. In the polluted Illinois River, almost unbelievable concentrations of bottom organisms have been found. How are such communities supported? What is the magnitude of primary production in comparison to other communities? How does the dominating current-flow relate to other energy-flows through the communities?

A large literature exists on the limnology of streams and the biology of flowing sea waters, but apparently studies on community function have rarely been oriented to obtain information on primary production (exceptions: Nusbaum and Miller 1952, Sargent and Austin 1949, 1954; Odum and Odum 1955, Purdy as quoted by Phelps 1944). This is very peculiar because the continual mixing makes *in situ* measurements of production very simple in flowing water. The task here, therefore, is to re-evaluate some of the extensive information on flowing waters in terms of productivity and community respiration especially by using diurnal gas curves and the upstream-downstream method of measuring community metabolism. A summary will then be made of the production and respiration of flowing communities relative to succession, velocity of current, and the classification of communities.

THEORETICAL CONSIDERATION OF DAILY PROCESSES OF OXYGEN METABOLISM IN FLOWING WATERS

Consider a stretch of flowing water delimited by two stations, one upstream from the other. During the usual daily cycle four main processes affect the oxygen and carbon-dioxide concentrations of water flowing between the stations. Although the discussion here is presented in terms of oxygen, it should be understood that carbon dioxide behaves similarly but with reversed sign.

(1) There is a release of oxygen into the water as a result of photosynthetic primary production during the day by both benthic plants and phytoplankton.

(2) There is an uptake of oxygen from the water as a result of the respiration of benthic

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organisms, planktonic organisms, and sometimes chemical oxidation.

(3) There is an exchange of oxygen with the air in a direction depending on the saturation gradient.

(4) There may be an influx of oxygen with accrual of ground water and surface drainage along the stretch. In most of the examples discussed here, accrual is assumed to be negligible relative to the other influences.

These processes between stations may be quantitatively summarized on an area basis (i.e., $g/m^2/hr$) as follows:

$$Q = P - R + D_{in} + A \qquad (1)$$

Q	P	R	D_{in}	A
Rate of change of dissolved oxygen per area	Rate of gross primary production per area	Rate of respiration per area	Rate of oxygen uptake by diffusion per area	Rate of drainage accrual

Note that capital letters have been used for these quantities that are defined on an area basis.

By dividing through by the depth in meters (z), the relationships are expressed in concentration-units (i.e., $g/m^3/hr$). Small letters have been used for these quantities which are defined on a volume basis.

$$q = p - r + d_{in} + a = \frac{Q}{z} \qquad (2)$$

The usual diurnal (24 hours) course of change for the component processes is given in Figure 1 for a hypothetical flowing system without accrual, which is both oversaturated and undersaturated with oxygen relative to the atmosphere and which has a balance of production and respiration. The photosynthesis-curve has the same shape as the incident light with a morning and afternoon symmetry as shown in Figure 1 for a clear day unmodified by cloud patterns.

If the plankton and biochemical oxygen-demand in the inflowing water remains fairly constant as assumed for the case in Figure 1, then respiration remains relatively constant. Whether plant respiration is depressed during photosynthesis or not is still an unsettled question since evidence is conflicting (Rabinowitch 1951, Whitting-

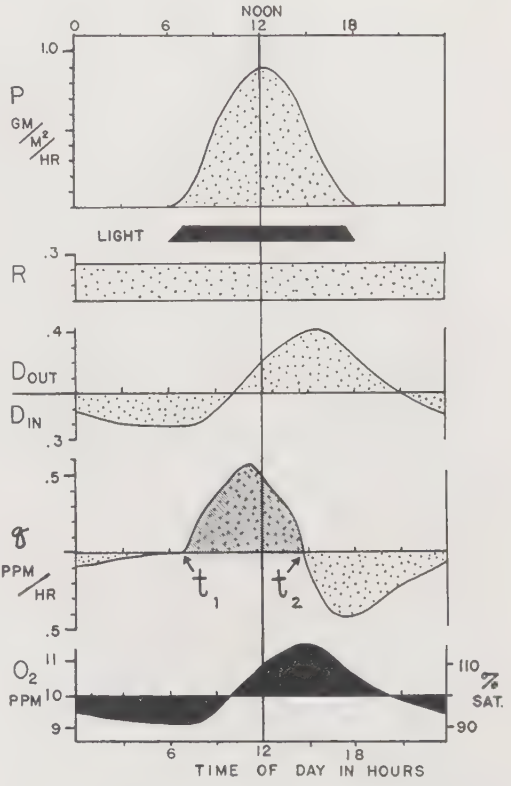

FIG. 1. Component processes in the oxygen metabolism of a section of a hypothetical stream during the course of a cloudless day. Production (P), respiration (R), and diffusion (D) are given on an areal basis. The combined effect of these rate processes for a stream 1 meter deep is given in ppm/hr (q). In the lowermost curve are given the actual oxygen values that would result in a stream with a long homogeneous community. The diffusion curve is linearly proportional to the oxygen concentration curve. The shaded area delimited by t_1 and t_2 is used in Equation (5) to obtain an approximate production estimate uncorrected for diffusion.

ham 1955). In strongly polluted waters with very low oxygen concentrations there may be a diurnal increase in respiratory oxidation during the day due to the formation of photosynthetic oxygen, where at night there is no oxygen. Respiration was found to be dependent upon oxygen tension below 1 ppm for sewage by Pomeroy (1938) and for marine waters by ZoBell (1940). Jackson and McFadden (1954) found an increase in community respiration during the day in lake waters. Until more is known about these and other diurnal effects, it may

be simplest here to continue to assume constant community respiration day and night. This assumption will not apply to flows a short distance below pollution outfall, where the volume of pollution discharge itself is frequently in diurnal cycle so as to cause minimum conditions of oxygen during the day (Schmassmann 1951, Calvert 1932, Mohlman *et al.* 1931).

Ordinarily the accrual of water is small and without daily variation. This is the case in Figure 1 where zero accrual is assumed.

Two recent reviews (Haney 1954, Ammon 1954) summarized knowledge on gaseous transfer into water. The rate of diffusion depends on the degree of saturation of the inflowing water.

$$D = KS = zkS \qquad (3)$$

where D is the diffusion rate per area, S is the saturation deficit between water and air, z the depth, K the gas transfer coefficient defined on an area basis (i.e., g/m^2/hr for 0% saturation) and k the gas transfer coefficient defined on a volume basis (i.e., g/m^3/hr for 0% saturation). The diffusion-curve sketched in Figure 1 was drawn for the case where the inflowing water had a similar history to the water preceding it because of similar conditions upstream. In this case an accumulation of oxygen develops in the water as production exceeds respiration during the morning so that diffusion into the water is replaced by diffusion out as the water becomes supersaturated. During the course of the day the other factors affecting diffusion rate such as depth, velocity, and bottom character tend to remain constant for the section of flow. Stream flows with large temperature ranges and changes in stratification-tendency may be exceptions.

When each of the component rates (P, R, D) given on an area basis are summed as in Equation (1), the total rate of change of oxygen is found for the zone between the two stations on an area basis. If this sum (Q) is divided by the mean depth (z) the rate of change of oxygen concentration is obtained (q), as in Figure 1 for a 1-meter deep flow.

From this rate-of-change curve of oxygen one may construct the curve of oxygen concentration by adding successive hours of change to the oxygen concentration at each hour. Thus, beginning with simple assumptions a diurnal oxygen curve for a stream was constructed (Fig. 1) to illustrate some of the known workings of stream communities. The oxygen curve obtained is typical of the curves observed by authors whose work was examined (see Table 2, also Cerny 1948, Wiken 1936). A morning maximum in the rate-of-change curve and an afternoon maximum in the oxygen-concentration curve are typical. Maximum rate of oxygen decrease occurs after sunset. Minimum oxygen concentration and minimum rates of change occur in the predawn hours. In the next paragraphs the procedure of reversing this analysis is described so that primary production may be estimated from observed oxygen curves.

MEASUREMENT OF METABOLISM IN STREAMS

Measurement of gross primary production

The light-dark bottle method for measuring gross primary production of the community (defined as the sum of the net plant production and community respiration during the daytime) is seldom applicable in flowing waters because much of the community is benthic and heterogeneous rather than planktonic. Furthermore, any measurement made without the normal turbulent flow may be questioned on grounds that production is a function of current flow. The upstream-downstream measurement of oxygen, carbon-dioxide, and other properties is apparently the chief method available for the study of metabolism of flowing water communities.

If the area under the upstream-downstream rate-of-change graph (q in Fig. 1) above a horizontal line drawn through the predawn point is measured, a first approximation to daily, gross primary production is obtained in concentration units (ppm/day). By this procedure one subtracts the accrual, respiration, and diffusion of the predawn hours from the rest of the curve as though these predawn values were representative of the entire day. Where the

change in oxygen concentration during 24 hours is small, so that diffusion is not markedly different between day and night, this approximation will give production values close to but always less than the actual production values. Therefore, the area under a rate-of-change curve of oxygen q (Fig. 1) expressed in ppm/day multiplied by the mean depth between stations (z in m) gives the gross production per area in g/m²/day (P).

The following is an alternative calculation where the oxygen change is expressed as the difference between stations rather than as the change per hour. The area under a curve of oxygen change between two stations (Δc) expressed in ppm-hours, multiplied by the discharge in m³/hr (F) and divided by the area between stations in m² (X) also gives the gross primary production per day (P).

These conversions may be summarized as follows:

$$P_{\text{day}} = z \int_{t_1}^{t_2} (q - q_1)\, dt = \frac{F}{X} \int_{t_1}^{t_2} (\Delta c - \Delta c_1)\, dt \qquad (4)$$

where t_1 is sunrise and t_2 is the time in the evening when q or Δc returns to its value at sunrise q_1 or Δc_1 respectively.

Or as measured in practice:

$$P_{\text{day}} = (z)(\text{area under diurnal } q \text{ curve}) = \frac{F}{X} (\text{area under diurnal } \Delta c \text{ curve}) \qquad (5)$$

These areas are hatched in Figures 1, 2, and 3.

The first of the calculation methods is used in the dye spot example for the marine turtle grass (Table 2) and in the analysis of single-station oxygen curves from the literature. The second of the calculation procedures is used in the Silver Springs example.

These simple methods without diffusion-corrections were used by Sargent and Austin (1949, 1954) in coral reef work at Rongelap where deviations from saturation were small relative to the total production.

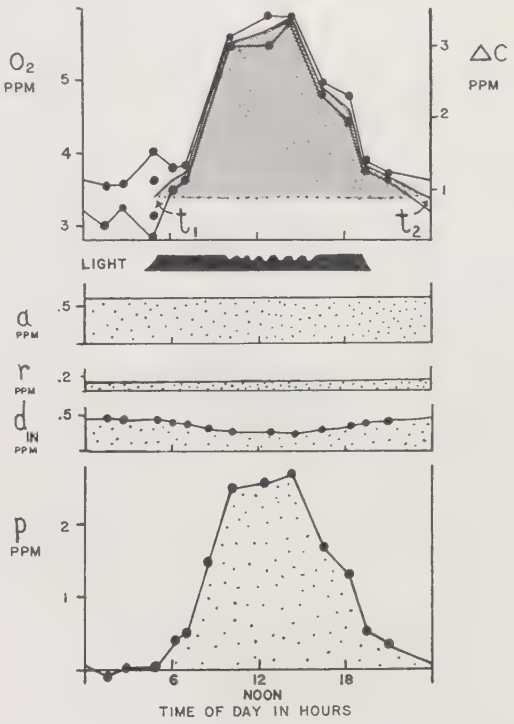

FIG. 2. Component processes in ppm in the daily oxygen metabolism in the upper ¾-mile zone of Silver Springs, Florida, March 23–24, 1954. In the upper graph duplicate oxygen analyses ¾ mile downstream from the outflow boil are expressed in ppm on the left ordinate. The ordinate on the right indicates the oxygen change (Δc) relative to the constant boil value of 2.50 ppm. The accrual (a) due to addition of more oxygenated water from side springs, respiration (r) as estimated with black belljars, and the diffusion in (d) as estimated from the gas transfer coefficient and the saturation deficit are given next. In the lower curve the production in ppm is computed by separating accrual, respiration, and diffusion from the observed oxygen change in the ¾-mile zone. The area under the lower curve expressed in ppm-hrs may be converted into daily production as indicated in Equation (5). The shaded area in the upper curve delimited by t_1 and t_2 is used in Equation (5) to obtain an approximate estimate of production uncorrected for diffusion.

It should be noted that the magnitude of diurnal change in ppm oxygen concentration gives no indication of the metabolic rates per area unless depth and velocity or discharge and area between stations are known. Small oxygen changes in deep or rapid streams may indicate larger production values than do larger oxygen changes of oxygen in shallow streams.

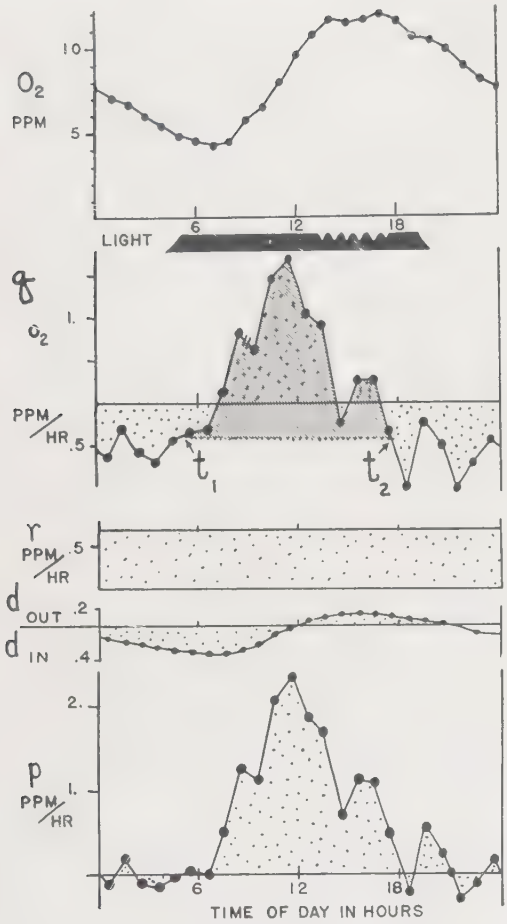

FIG. 3. Component processes in ppm/hr in the daily oxygen metabolism of the River Lark, England, calculated by the single-curve method from data given by Butcher, Pentelow, and Woodley (1930). In the upper curve is given the observed diurnal (24-hr) oxygen curve. From this is calculated the rate of change per hour in ppm/hr (q). Respiration (r), diffusion (d), and production (p) on a ppm-basis are calculated as indicated in the text on the assumption that the stream is homogeneous. The shaded area delimited by t_1 and t_2 is used in Equation (5) to determine the approximate production uncorrected for diffusion.

As considered so far these calculations of gross primary production do not consider diurnal variations in respiration, accrual, and diffusion. Since diffusion ordinarily is the main process which fluctuates diurnally, the success and reliability of the upstream-downstream method is dependent upon the accuracy of corrections for diurnal diffusion-change.

Measurement of diffusion

Especially where differences in rate of diffusion between night and day are large, such as in the diagrammatic example in Figure 1, it is necessary to estimate the change in diffusion due to the daily change in saturation deficit. If a coefficient of gas-transfer (K) is available or can be obtained for the stream section in question, it may be used to apply a correction for each hour of the day as in Figures 2 and 3 according to the saturation deficit for each hour.

In Table 1 are given various estimates of K for oxygen transfer in flux units. Values for still water with mostly molecular diffusion are about 0.03 g/m²/hr/0.2 atm. Values for bubbles and droplets as in waterfalls are as high as 34 g/m²/hr/0.2 atm. The values for flowing waters are in between, depending upon the rate at which the surface film is dispersed downward due to turbulent mixing.

The following procedures can be used to measure the gas transfer coefficient in flowing waters:

(a) The gas transfer coefficient may be obtained from two measurements of oxygen content of the water, one after sunset and one just before sunrise.

According to the relationships in equations (2) and (3) before sunrise:

$$q_m = \frac{KS_m}{z} - r \qquad (6)$$

where S_m is the predawn saturation deficit and q_m is the rate change of oxygen concentration in the morning. After sunset:

$$q_e = \frac{KS_e}{z} - r \qquad (7)$$

where S_e is the evening saturation deficit and q_e is the rate of change of oxygen concentration in the evening.

Subtracting these expressions leads to:

$$K = \frac{z(q_m - q_e)}{S_m - S_e} \qquad (8)$$

The gas transfer coefficient can be readily obtained from diurnal oxygen curves by

TABLE 1. *Coefficients of gas transfer for oxygen*

Water type, Source of data	Velocity m/sec	Depth m	Temp. °C	K g/m²/hr at 0% saturation
Still water				
(Hutchinson, Becker, quoted by Haney, 1954)	0.0	—	20–25	.034
(Adenay, quoted by Kehr 1938)	0.0	—	—	0.03–0.08
Moving water				
Stirred water (quoted by Haney 1954)	—	—	25	0.09–0.74
Shallow circulating trough (Streeter, Wright, and Kehr 1936)	0.01	0.1	0–10	0.037
	0.01	0.1	10–20	0.043
	0.01	0.1	20–30	0.047
	0.013	0.1	12	0.12
	0.070	0.1	17	0.52
	0.119	0.1	14	1.12
	0.20	0.1	13	3.8
Sewage in circulating trough (Kehr 1938)	0.05	0.45	25–26	0.38
	0.15	0.45	25–26	1.5
Stream and ponds (Imhoff and Mahr 1932)	—	—	—	0.08
New York Harbor (Gould 1921)	tidal	—	—	0.23
Tank with a wave machine (Borst, quoted by Phelps 1944)	—	1.8	— —	0.31
Sea Surface (Redfield 1948)				
Summer	—	—	12–20	1.1
Winter	—	—	3–7	5.2
Silver River, Florida, July 21, 1955 (Odum)				
Subtraction-of-respiration method	0.21	2.77	23	0.92
Dye-measured-turnover method	0.21	2.77	23	1.00
Green Cove Springs, Florida (Odum) From carbon-dioxide by respiratory-quotient method	0.3	0.23	24	0.55
Small rivers, diurnal oxygen curve analyses in Table 2	—	0.5–3.	—	0.6–4.3
Ohio River below Cincinnati (Velz 1939)	0.05–0.09	4.8	15–25	1.5–5.0
Bubbles and drops (K given per area of drop or bubble)				
Air bubble (Krogh, quoted by Redfield 1948)	—	—	37	13.1
Air bubbles (Ippen, Adenay, Spuler, and Schwab, quoted by Haney 1954)	—	—	20–25	2.8–28.
Water drops (Whitman, quoted by Haney 1954)	—	—	24	22–34.

substitution of measured values in Equation (8).

The rapid production of gases in some cases produces local extreme supersaturations so that bubbles break to the surface without going into solution even in undersaturated waters. Measurements by trapping bubbles in funnel devices showed about 1.5% of the production or 0.3 g/m²/day loss during the daylight hours in Silver Springs. The effect is more important in shallower streams. Even though the rate is partly a function of the saturation deficit, it ceases as an oxygen-loss mechanism at night. Thus, diffusion coefficients determined at night may fail to include this effect. In shallow supersaturated waters this loss leads to underestimation.

(b) If some estimate of the time required for vertical mixing and dispersion of the surface film is available, the coefficient of diffusion may be computed on theoretical grounds (Phelps 1944, after Black and Phelps 1911). In Silver Springs fluorescein dye was observed from under water with a face mask. The yellow surface film was readily observed to become dispersed vertically in about 1 minute. The calculated gas transfer constant was similar to the inferred value from balance sheet calculations (Table 1).

(c) If a respiration value has been obtained for a stream and if accural is negligible, the diffusion rate for a known saturation deficit may be obtained by subtracting the respiration from the upstream-downstream change at night. For example, belljar measurements were used to obtain an estimate of respiration in Silver Springs. The diffusion was then calculated (Table 1).

(d) If the diffusion rate is found for one gas (i.e. CO_2) the diffusion rate for another gas may be related as their molecular diffusion coefficients. When transfer coefficients are expressed in $g/m^2/hr/atmosphere$ saturation-deficit, similar values are found for both gases.

(e) If river slope, velocity, and depth are known, some idea of K may be obtained by using empirical equations based on observations on the Ohio River (Phelps 1944, Streeter and Phelps 1925).

(f) In a section of a river without accrual the difference between gross primary production and respiration, expressed in organic matter, is the organic matter synthesized in this section. P-R is readily measured by upstream-downstream organic matter measurements. Substitution in Equation (1) permits the calculation of the diffusion. This method assumes that sedimentation is balanced by erosion, as in the middle sections of many rivers.

(g) Lacking other means a value may be selected from Table 1 for similar conditions of depth and turbulence.

Measurement of community respiration

Where community respiration is to be measured, the following procedures are available:

(a) Black belljars over typical bottom-substrates and black bottles in the water may be combined to obtain respiration rates. In many streams the black bottle respiration-rate differs from the stream respiration due to differences in bacteria, turbulence, oxygen conditions, and benthic communities. Similar difficulties arise with attempts to anticipate the natural black bottle measurements with BOD tests of pollution effluents.

(b) During darkness, the diffusion may be subtracted from the observed rate of change to obtain the respiration in ppm. The ppm/hr respiration multiplied by the depth in meters gives the respiration per area per time in $g/m^2/hr$. If during darkness there is a time of saturation with respect to the atmosphere, such as occurs after sunset in Figure 1, the upstream-downstream change is equal to the respiration providing the accrual is negligible.

(c) If one gas (i.e. CO_2) is nearly at saturation, and its respiration component can thus be obtained at night, the respiration of the other gas (i.e. O_2) may be obtained assuming a respiratory quotient characteristic of the stream or arbitrarily 0.8 as an approximation.

The methods suggested here for measurement of diffusion, respiration, and photosynthesis are demonstrated in subsequent paragraphs with three examples representing three important, frequently occurring cases.

An example of production measurement in constant inflow situations

Silver Springs, Florida, is an example of chemostatically regulated inflow. Since there is no diurnal change in the upstream station (the boil outflow), the downstream concentration curve is readily converted into an upstream-downstream rate of change curve by subtracting the upstream value from each downstream value and dividing by the time of flow to the downstream station. In Figure 2 is shown the oxygen concentration curve, upstream-downstream change curve, and component respiration, accrual, and production curves based on detailed work presented elsewhere (Odum 1954). The accrual is large and the zone is always undersaturated. Respiration was measured by belljar measurements of the benthic communities, and diffusion estimated as indicated in Table 1. Possible errors in this method were cited previously.

Other flows with fairly constant inflow without much diurnal oxygen variation are found downstream from large waterfalls, in ground water flows, and downstream from surf. The Rongelap and Eniwetok coral reef studies include measurements under situations of relatively constant inflow (Sargent and Austin 1949, 1954; Odum and Odum 1955).

Production measurements by the difference between upstream and downstream diurnal curves

For the usual complex case where no simplifying assumptions may be made about the inflowing water, complete curves should be taken at two stations. Upstream-down-

stream changes were used in the coral reef studies of Sargent and Austin (1949, 1954) and Odum and Odum (1955). The upstream and downstream curves in Schmassmann (1951) are examples of data to which this analysis might be applied if information on depth and flow rate were available. The upstream curve can be subtracted from the downstream curve after shifting the upstream curve to the left by a time period corresponding to the time required for the flow to pass from the first to the second station. Unfortunately, most of the diurnal oxygen and carbon-dioxide curves in the literature are from single stations.

An example of production measurement by single curve analysis

Where a curve for only one station is available, but an assumption of stream homogeneity above the zone of measurement is reasonable, the curve for rate of change may be obtained from a single diurnal oxygen graph. With this procedure one assumes that the incoming water had had the same diurnal history as the water just preceding. In other words the whole stream is experiencing a simultaneous rise and fall of oxygen. In a stream where this assumption applies perfectly, a second station would reveal a curve identical with that of the first station. The assumption is consistent with the theoretical case of a circular stream and a homogeneous community. Most of the data discussed in this paper are based on curve-analysis of single curves.

In Figure 3 the oxygen curve, rate-of-change curve, and resulting component curves of respiration and diffusion are shown for the River Lark based on data given by Butcher, Pentelow, and Woodley (1930). It should be emphasized that this single-curve procedure is no substitute for a double-curve analysis, but is useful in obtaining orders of magnitude. A partial curve analysis was used by Jackson and McFadden (1954) for measuring production in a lake.

Spot method

A very simple method used by the author and J. Yount for obtaining upstream-downstream changes in flowing water is outlined as follows: A spot of fluorescein dye is placed in the water and followed for about 15 minutes. Measurements are taken in water adjacent to the marker spot before and after the period. Then the observers return upstream to the starting point before making the second measurement. In this way a diurnal rate of change curve is obtained which is then analyzed to obtain diffusion, respiration, and production as described above. This method was used for a bed of marine turtle grass (*Thalassia*) in 3 ft of water along the causeway at Long Key, Florida (Table 2). This is probably the simplest of all the methods described here.

PRODUCTION AND COMMUNITY STRUCTURE OF STREAM COMMUNITIES

Magnitudes of primary production in streams

Using the methods described in preceding sections, data from the literature have been used to obtain the estimates in Table 2. For comparison recall that eutrophic lakes have a gross primary production of organic matter of the order of magnitude of 1 (0.5–5) g/m^2/day (Gessner 1949) during seasons of maximum growth; oceanic waters have gross production magnitudes of 0.17–1.6 g/m^2/day (Riley 1953); terrestrial agriculture under the best circumstances achieves sustained net production of 10–20 g/m^2/day (Kalle 1948); and mass *Chlorella*-cultures under strong light and bubbling carbon-dioxide yield a net production of 2–19 g/m^2/day (Wassink, Kok, and Oorschot 1953). If the ratio of photosynthesis to respiration is about 2.0 this indicates a maximum gross primary production of *Chlorella* of 28 g/m^2/day. It is immediately observed that the estimates in Table 2 for production in flowing waters are very high. It may be suggested that streams are among the most productive biological environments, a conclusion that has been suspected for a long time on the basis of estimates of standing crop.

Comparison among the streams indicates that the highest primary production rates are in the recovery zones of streams polluted with organic wastes. Thus the pollution not only increases the biota due to an additional organic nutritive source, but subse-

TABLE 2. *Productivity and classification of flowing water communities on the basis of oxygen metabolism data*

Stream, Date, Source	*P* Gross production g/m²/day	*R* Community respiration g/m²/day	*P/R*	*K* Gas transfer coefficient g/m²/hr at 0% saturation
Autotrophic communities ($P/R > 1$)				
Windward coral reef, Eniwetok, July, 1954 (Odum and Odum 1955)	24	24	1	—
Silver Springs, Florida (Odum) respiration from bell jars				
Winter, 1952, 1953	8	2.8	2.9	—
March 23–24, 1954	35	5	7.0	0.92
Birs, Switzerland (Schmassman 1951)[1] April 11–12, 1946	50	18	2.8	3.3
White River, Indiana; zone of recovery from pollution (Denham 1938)[1] July 23–24, 1933	57	18	3.2	1.6
Kljasma, Russia (Brujewica 1931)[1] July 21, 1929	2.4	1.9	1.3	0.6
10 Florida Springs (Odum) July, August, 1955	0.6–59.	—	>1	—
Turtle Grass, Long Key, Florida (Odum and Yount) August 14, 1955	34.	24.	1.4	2.6
Heterotrophic communities ($P/R < 1$)				
Itchen River, England; unpolluted (Butcher, *et al.* 1930)[1], Figure 4				
April–October	5.5–14.0	5.8–18.6	0.6–1.1	0.9–2.8
November–March	0.4–7.1	4.2–20.2	0.1–0.5	1.5–4.3
River Lark, England; polluted (Butcher *et al.* 1930)[1]				
November 17–18, 1927	0.53	53	0.01	—
May 18–19, 1927	39.	35	1.1	2.3
White River Indiana, near pollution outfall (Denham 1938)[1] July 21, 1934	0.24	29	0.008	1.22
Potomac Estuary; balance sheet calculation by Purdy, 1917 (quoted by Phelps 1944), eelgrass; polluted	11.0	16.8	0.66	3.7
San Diego Bay, black bottle method; production estimates of Nusbaum and Miller (1952)	2.8	4.4	0.65	1–2.

[1] Diurnal oxygen curves from the paper cited were used as described in the text to obtain production, respiration, and gas transfer estimates.

quently increases the autotrophic based growth rates.

High production is not limited, however, to polluted waters. Three flows with the lowest organic matter contents of all waters (0.1–1 ppm)—the Pacific coral reef environment, the Caribbean turtle grass and the large artesian calcareous springs in Florida—possess very high productivities (Table 2).

The seasonal course of gross production and community respiration has been calculated for the unpolluted River Itchen from data given by Butcher, Pentelow, and Woodley (1930) and summarized in Figure 4. Each point was obtained with a curve analysis like that in Figure 3. An approximation was introduced in the calculations by the use of the average depth 0.6 m rather than the river stages on the particular days, which were not readily available. The production curve shows a seasonal trend correlated with the course of sunlight. That the light is the main cause of this pattern seems likely. A similar curve was found in the constant temperature of Silver Springs (Odum 1954). Verduin (1956) found that naturally adjusted plankton populations had a primary production independent of temperature. The constancy of respiration in Figure 4 is supported by Butcher, Pentelow, and Woodley's (1930) account of fairly constant benthic plant populations during the year in the River Itchen. That similar values of the gas transfer coefficients were obtained (Table 1) in spite of the depth approximation used, gives one some confidence in the single-curve analysis procedure used. The seasonal shift in K values for a given stream may not be more than 2- or 3-fold.

Although the downstream longitudinal

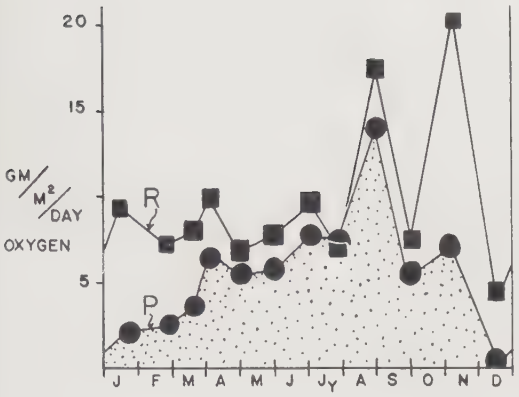

FIG. 4. Seasonal course of production (P) and community respiration (R) for the Itchen River, England, estimated with the single-station method from data given by Butcher, Pentelow, and Woodley (1930). Production per day in ppm was converted to an areal basis using an approximate depth of 0.6 meters. The stream was unpolluted, calcareous, and contained large plant populations.

succession of species, physical conditions, and rates of waste decomposition are now well-known and fairly similar throughout the world where studied, almost no effort has been made to estimate the primary production rates in the recovery sequence below sewage pollution discharges. Using some oxygen curves of Calvert (1932, 1933) and Denham (1938) for the White River in Indiana, the single-curve procedure has been used to obtain the longitudinal distribution of primary production and respiration over the 160 miles below the Indianapolis waste outfall. As shown in the pattern in Figure 5, respiratory metabolism far exceeds production in the first 20 miles as the sewage decomposes. Production, however, rises rapidly so that primary production far exceeds the decomposition processes in the early recovery zone. Here the organic matter is being made faster than it is being used. The rise in respiration that seems to occur below this point may be a result of the increased organic matter and increased populations of respiratory organisms. This is in keeping with the idea that the respiratory metabolism of a polluted stream is proportional to the organic matter content at a rate determined by the deoxygenation constant (Streeter 1935, Velz 1939, Phelps 1944). The second peak of respiratory metabolism 100 miles downstream is accompanied by aerobic daytime conditions although the diurnal varition is large. A supersaturated oxygen condition during the day and an anaerobic condition at night is a property of such waters. For example, Schmassmann (1951) used the range of oxygen concentration to classify such streams. Low oxygen and high organic matter are therefore not always correlated. Far downstream there is a gradual decline of both production and respiration. The pattern of maximum primary production in the early recovery zone fits the observed maxima of algal populations in this zone as observed,

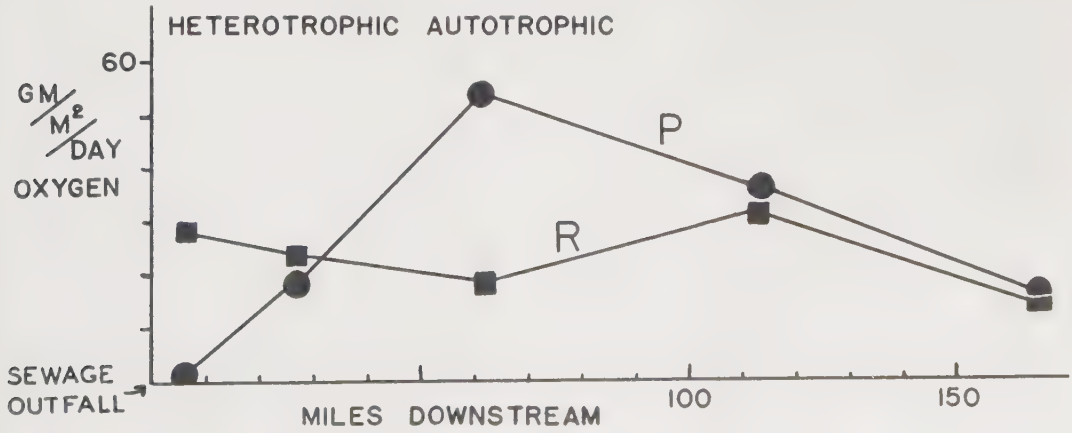

FIG. 5. The downstream sequence of production (P) and community respiration (R) estimated with the single-station method from data given by Denham (1938) below a sewage pollution outfall in the White River, Indiana, July 21–27, 1934.

for example, in the River Trent by Butcher (quoted by Macan and Worthington 1947).

Enormous values of primary production are also found in recovery zones of other polluted streams as shown in Table 2 and including the River Lark, England, and Birs, Switzerland. It may be found eventually that the most productive communities in existence are those in polluted streams.

Efficiency and current velocity

The estimates of high production in Table 2 for flows of many kinds suggest some consistent difference between the production in running waters and the production in other environments. One is tempted to conclude that the efficiencies of primary production in streams are higher than those of most other environments.

Although many streams maintain higher nutrient concentrations than still waters, this cannot be the entire reason for the high production. The high production-measurements in extremely low nutrient waters at Eniwetok and at Rongelap indicate that nutrient concentration can be overcome where current velocities are large and the organisms are adapted. Ruttner in 1926 (Ruttner 1953) showed that a flow of current past a community accelerates those metabolic processes that are limited by slow rates of diffusion. The current renews the depleted requirements for life and removes accumulating by-products of metabolism. Many organisms adapted to such currents cannot survive outside of the currents. The importance of this phenomenon has been recognized in some small invertebrate animals in their current demand and the high metabolic rates in comparison to similar lake species. Munk and Riley (1952) have made computations as to the effect of falling plankton of various shapes in stimulating their own metabolism by moving relative to the water.

Organic matter, productivity, and succession

In flowing systems there are three time sequences in the adjustment of communities to the physical environment. One is the sequence at one place in the stream, the temporal (short term) succession. The second is the action of a community upstream in determining subsequent events downstream by affecting the contents of the water passing downstream (the longitudinal succession). The third is the long-time geological succession by which erosion transforms "V"-shaped valley headwaters into flood-plain physiography. Long-time geological processes will not be considered further here. Considerable opinion and evidence exists that temporal succession rapidly leads to an aquatic climax (Butcher, 1945, 1949; Shelford and Eddy 1929), which is maintained until the properties of the water-flow change. Similarly, detailed studies have shown sequences of longitudinal succession such as the pollution-recovery sequence already considered, the cooling sequence downstream from hot springs (Seurat 1922, quoted by Hesse, Allee, and Schmidt 1951), or the diversification sequence downstream from cold springs (Sloan 1956). Although data are scarce, some basic considerations of the production processes may help to relate production to successional sequences.

In Figure 6 is given an energy flow diagram for a community composed of the 5 trophic levels: primary producing plants, herbivores, decomposers, carnivores, and top carnivores. The diagram is drawn so as to separate the photosynthetic part of the community from all the respiratory parts of the community including the plant respiration. Following the first law of thermodynamics the rates of influx of both light and organic matter energy sources are equal to the energy outflux of heat and organic matter. According to the second law of thermodynamics, any process that takes place spontaneously involves a heat loss (dispersal) as an entropy tax. From this diagram it should be clear that when subsequently gross production, net production and respiration are used, community values are intended rather than physiological values.

In many stream communities, there is a large influx of imported organic matter. The word export is appropriate for the organic matter that leaves an active community either by being sent downstream or sedimented below the active metabolic level

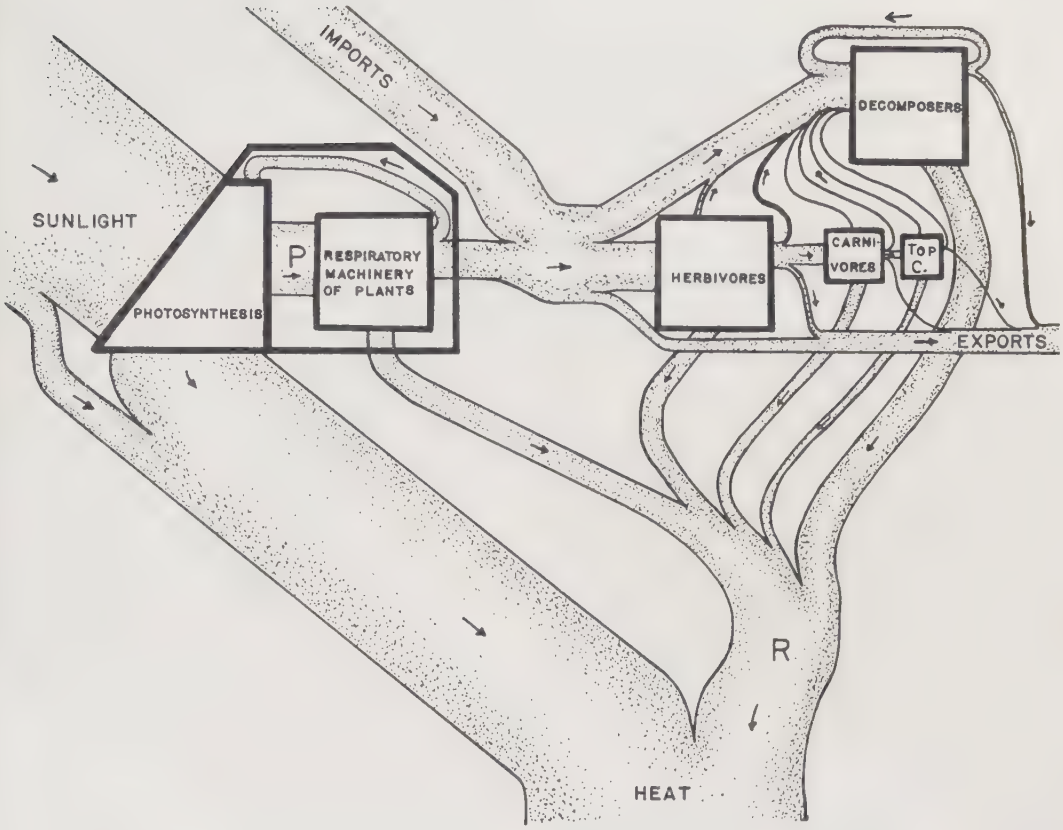

FIG. 6. A generalized diagram of energy flow for steady-state natural communities. P is the gross primary production; R is the total community respiration; the 5 trophic levels are indicated by boxes. Imports and exports are included as dominant flows which may equal the flow of primary production in some streams. The flow from the top of those boxes representing consumer trophic levels indicates unassimilated materials. The laws of thermodynamics are illustrated since inflows balance outflows and every process is accompanied by dispersion of heat energy as entropy tax.

(into a condition of low oxidation potential) of the community. Thus for the whole flowing community:

$$I_m + P = E_x + R \qquad (9)$$

where I_m is the import rate, E_x the export rate, P the production rate, and R the community respiration rate. The quantity of consumers that can be supported is dependent both on the primary production and the import.

There seems to be inherent in these arrangements a self-regulating mechanism that tends to direct longitudinal succession but not succession *in situ*. If as cited above for polluted streams, the respiration is a function of the concentration of organic matter in the water and on the bottom, while the primary production is a function of light and nutrients, there is possible an organic matter concentration in the water at which production will balance respiration. For concentrations of organic matter below this there will be too little available free energy in organic matter to build large populations of organisms. In these cases production will exceed respiration and there will be a net storage or export of organic matter. On the other hand when respiration exceeds production there will be a net loss or import of organic matter. The types of standing crop characteristic of these regions may show dramatic differences. Silver Springs, Florida, is an example of a community that has

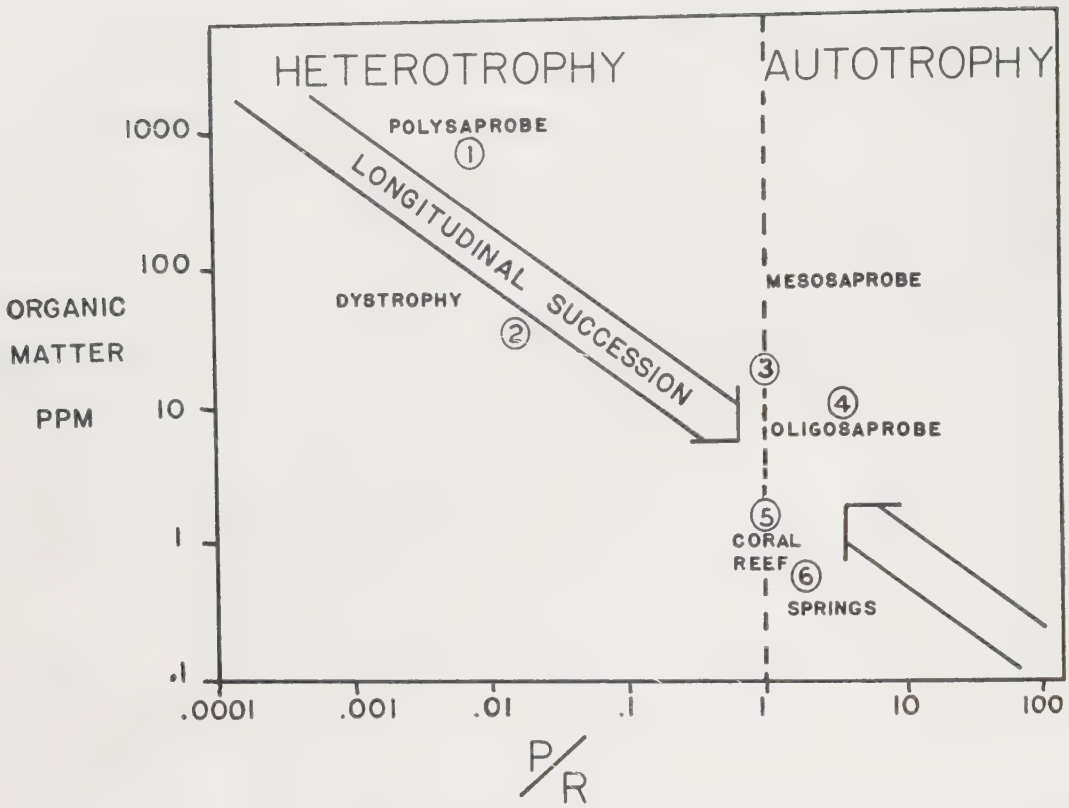

FIG. 7. The relationship of longitudinal succession, total organic matter in waters, and the relative dominance of autotrophic and heterotrophic metabolism as indicated by the P/R ratio. The arrows indicate the trend of concentration of organic matter in waters, depending on the P/R ratio of the communities over which the water flows. Several specific communities are plotted: (1) White River pollution outfall (see Fig. 5); (2) an approximate point for a dystrophic stream based on the observation that the oxygen tension remains below saturation (3.5 ppm) both day and night (Santa Fe River, Fla., Oct. 31, 1953); (3) recovery zone of the Illinois River at Averyville, Aug. 24, 1927, from single station calculation of diurnal oxygen curve given by Mohlman *et al.* (1931); (4) Birs, Switzerland (Table 2); (5) coral reefs from Sargent and Austin (1948, 1954), and Rongelap and Eniwetok from Odum and Odum (1955); (6) Silver Springs, Fla., annual means.

an excess of production over respiration so that the organic matter is exported and the particulate organic content of the water increases downstream. Thus longitudinal succession beginning with either high or low organic content will trend towards an intermediate organic matter content as a steady state downstream condition. Within the length of most streams there is not time for much succession. Many streams with large and continual allochthonous organic matter imports from land drainage will be in an arrested longitudinal succession. These concepts can be made operational if the ratio of production to respiration can be related to the concentration of organic matter in the water (expressed in mg/l). For natural summer light intensities the organic matter content corresponding to a P/R of 1 is of the order of magnitude of 1–15 ppm. Purdy (1935) found photosynthesis exceeded respiration in mixed algal and bacterial cultures containing 10 ppm dextrose and peptone. Many harmonic and unpolluted lakes and oceans tend to be maintained in this range (Thunmark 1937, Birge and Juday 1926). According to this thinking, deviations much below or above this range cannot occur without addition and removal of organic matter by other agencies, as in

ground water filtration or in allochthonous organic matter drainages. These trends are summarized in Figure 7.

Temporal succession in one place in flowing systems is unable to modify its own water environment, since its products are whisked away to cause downstream succession. Thus successional phenomena in streams are much more a function of the influx than in terrestrial or still water communities. It seems likely that there are a great many possible climaxes depending on the hydrographic climate provided by the inflowing water. A rational basis for classifying these climaxes in terms of production and respiration processes is provided by the P/R ratio.

The tendency for the P/R values to ap-

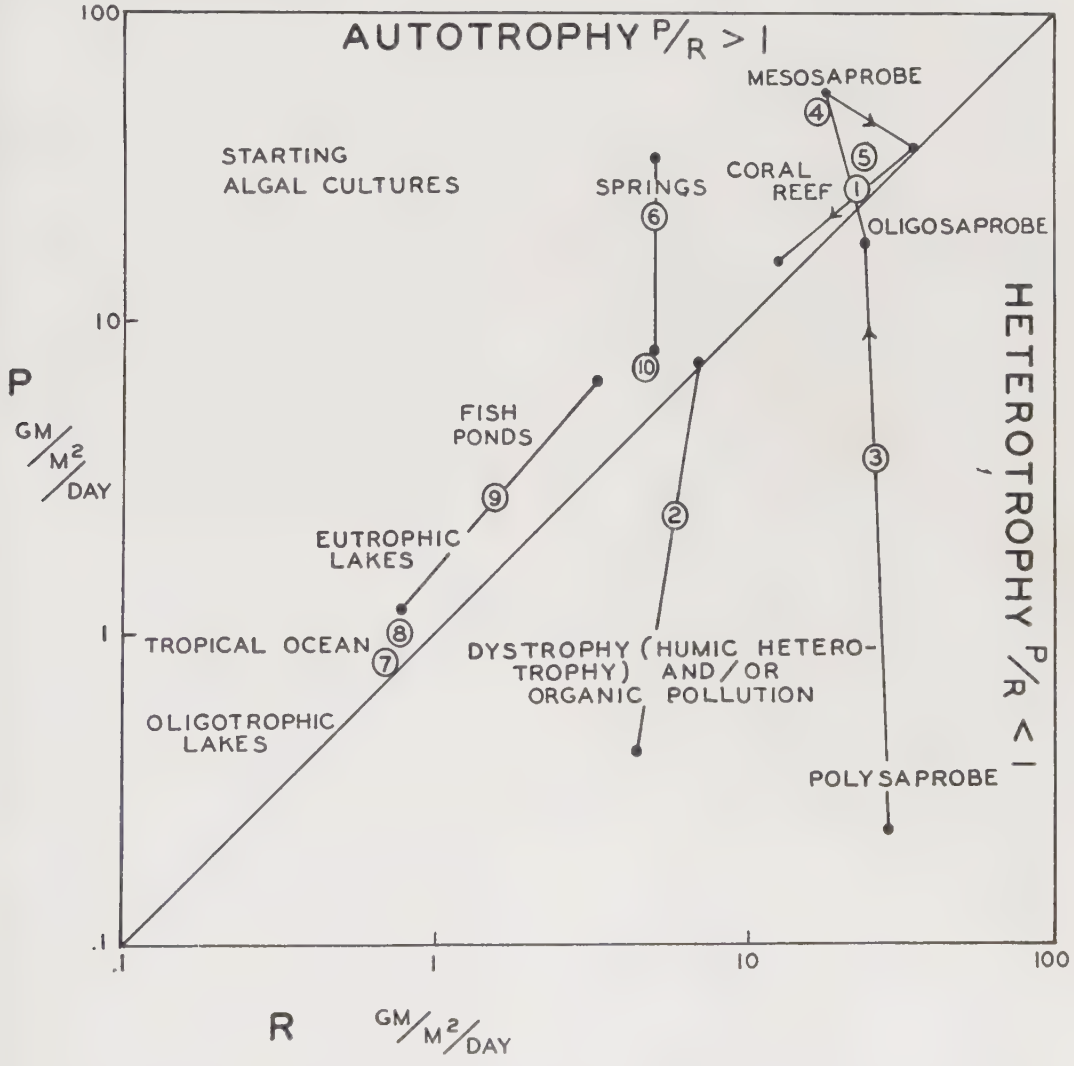

FIG. 8. A diagram for the functional classification of communities according to the total metabolism and relative dominance of autotrophic and heterotrophic metabolism. Specific examples are indicated as follows: (1) Coral reefs (Table 2); (2) Itchen River, England (Fig. 4 and Table 2); (3) recovery sequence on the White River, Indiana (see Figure 5); (4) Birs, Switzerland (see Table 2); (5) Turtle grass (see Table 2); (6) Silver Springs (see Table 2); (7) Tropical ocean (after Riley 1953); (8) Linsley Pond, Conn. (Riley 1940); (9) Little John Pond, Wisconsin (Juday, Blair, and Wilda 1943); (10) fish ponds (from Winberg, 1937, as quoted by Juday *et al.* 1943). Ryther (1954) described the behavior of the P/R ratio in algal cultures.

proach some moderate value is consistent with the qualitative description of rocky stream biota, which is dominated by algae in the headwaters and by consumer animals in the downstream sections. Pennak (1943) described a dominance of phytoplankton over zooplankton in the headwaters of a mountain stream. An amusing example was provided by Nikolsky (1937), who showed that fishes in the headwaters of the Amudaria River had gut lengths 235% of body length, decreasing to but only 170% of body length in the delta. The long guts are presumably a correlation of the plant-eating habits of the fish in the headwaters.

The P/R *diagram*

As described in the foregoing paragraphs the P/R ratio enables one to make a logical classification of communities into autotrophic and heterotrophic types. In Figure 8, P and R are used as coordinates of a diagram thus providing a useful graph for quantitative comparison of communities. The flowing water communities discussed in this paper are plotted along with some representative values from other environments. The diagram shows how some streams may be fertile in having high total respiratory metabolism and yet possess little primary productivity. It may be desirable to distinguish heterotrophic and autotrophic types of fertility with such terms as hetero-eutrophic, hetero-oligotrophic, auto-eutrophic, and auto-oligotrophic. The diagram suggests a quantitative definition of dystrophy as a term applicable to communities with humic-colored water and a P/R less than 1.

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Body Size and Number of Eggs in Diaptomids, as Related to Water Renewal in Mountain Lakes

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ABSTRACT

The study of 32 populations of *Arctodiaptomus bacillifer* and of 14 populations of *Acanthodiaptomus denticornis* has shown a good correlation between the body size of egg-carrying females and the number of eggs actually carried by the same. Furthermore, these characters are correlated with the extent of the water renewal of each lake, based upon the ratio: area of drainage basin/area of lake. It is suggested that in any population, selection may favor females able to produce a number of eggs correlated with and balanced against the losses which outflowing waters induce in the pelagic stages of the population.

Two species of diaptomids, *Arctodiaptomus bacillifer* Koelb. and *Acanthodiaptomus denticornis* Wierz., typically occur in the tarns at high altitude in the Alps. In lakes higher than 2000 m, the population is represented for a long period of time (from the beginning of autumn until the end of spring) by resting eggs, which develop into nauplii and then during summer into adults, and these in turn produce new resting eggs in late summer. Sporadically in warmer lakes, the same female can lay two successive egg-sacks, of which the first one can be composed of subitaneous eggs, which hatch and reach the first nauplius stages; but very seldom do the meteorological conditions occur that are necessary for completion of development, thereby producing a second generation in the same year.

It can be postulated that if in different lakes the same mortality rate were to occur during the whole development from the egg to the ovigerous female, we should observe a very similar number of eggs in the egg-sacks of different populations. On the contrary, it has been observed many times (Hutchinson 1951, Margalef 1953) that the number of eggs carried by females of the same species can be very different when distinct populations are compared.

Every breeding season along with its preceding more or less normal meteorological vicissitudes can affect the egg productivity, but we believe that it cannot obscure the differences typical for each lake, because of the very short period during which the populations are pelagic, and because of the fact that for these species of diaptomids strong competitors are not normally present.

Starting from these considerations we have examined 32 different populations of *Arctodiaptomus bacillifer* and 14 of *Acanthodiaptomus denticornis*. For each population the number of eggs contained in the egg-sacks of from 10 to 30 females was counted, depending on the size of the samples available. At the same time the length of the cephalothorax was measured, and the standard error was calculated for the mean of both values within each lake (Tables 1 and 2). The data suggest the existence of a relation between the body size of the ovigerous females within each population and the mean number of eggs carried by them. Indeed, the correlation analysis between these two variables gives $r = .634$ for *denticornis* and $r = .730$ for *bacillifer*, which correlations are significant beyond the .02 and .001 levels, respectively. The t-values for testing the non-casualty of the fact are 2.84 for *denticornis* (between the .01 and .02 levels of significance) and 5.85 for *bacillifer* ($<.01$). This permits one to state that any population requiring a high rate of natality in order to maintain approximately the same number of individuals year after year has an advantage in selecting bigger females.

A similar correlation was found in marine copepods by Marshall (1949) with a coefficient of .75 for *Oithona similis* and .98 for *Pseudocalanus minutus* (35 pairs of observations in both organisms).

The importance of outflowing water as a

TABLE 1. *Mean cephalothorax length and mean number of eggs for 32 populations of* Arctodiaptomus bacillifer *in high altitude tarns of northern Italy, arranged in order of increasing mean number of eggs per female*

Lake	Valley	Altitude (m)	Date	No. females	Length cephalothorax in μ (mean ± std. error)	No. eggs (mean ± std. error)
"Ai Laghetti" Lagorai	d'Avisio	2,266	22. 9.1931	13	818.32 ± 7.95	1.54 ± 0.14
Gelato	di Campiglio	2,386	1. 9.1949	30	998.07 ± 10.57	1.93 ± 0.10
Venerocolo	di Scalve	2,298	27. 8.1949	30	965.41 ± 7.75	2.47 ± 0.16
Coldai	del Cordevole	2,146	7. 9.1949	17	990.85 ± 12.00	2.94 ± 0.35
Bleu	Valtournanche	2,000	1921	30	881.29 ± 10.04	3.00 ± 0.19
Superiore Corno d'Olen	Valsesia	2,300	25. 8.1922	25	928.51 ± 11.78	3.20 ± 0.22
Paione Superiore	Bognanco	2,269	23. 9.1952	30	941.61 ± 7.71	3.30 ± 0.22
Erdemolo	dei Mocheni	1,994	7.10.1934	30	927.84 ± 11.16	3.53 ± 0.21
Monscera	Bognanco	2,071	30. 6.1947	10	859.87 ± 10.47	3.60 ± 0.37
Bundèn	Spluga	2,400	23. 8.1949	30	924.66 ± 6.51	3.97 ± 0.23
Paione Inferiore	Bognanco	2,002	23. 9.1946	30	1053.78 ± 7.59	4.33 ± 0.24
Nivolet Inferiore	Valsavaranche	2,526	21. 8.1949	21	1077.99 ± 13.76	4.62 ± 0.49
Superiore Alpe Ligher	Anzasca	2,550	8. 8.1947	19	908.01 ± 6.17	5.16 ± 0.35
Boden S	Formazza	2,343	21. 9.1954	28	994.54 ± 9.47	5.79 ± 0.41
Boden W	Formazza	2,313	21. 9.1954	30	887.52 ± 5.33	6.00 ± 0.28
Antermoia	di Fassa	2,496	1. 9.1935	30	948.47 ± 17.24	6.17 ± 0.29
del Coston	Solda	2,651	30. 7.1949	17	972.87 ± 19.84	6.18 ± 0.47
della Mulattiera	Valsesia	2,370	25. 8.1922	22	1013.63 ± 13.83	6.32 ± 0.19
delle Valli Superiore	di Scalve	1,971	8. 8.1949	11	1164.77 ± 10.17	6.45 ± 0.49
al Rifugio Denza	di Sole	2,298	31. 7.1949	17	1121.32 ± 11.30	7.23 ± 0.45
d'Agro	Bognanco	2,041	23. 9.1952	30	1010.93 ± 6.76	7.87 ± 0.19
Lavazza	Belviso	2,300	29. 7.1949	30	977.99 ± 7.94	8.23 ± 0.25
Paione Medio	Bognanco	2,147	21. 9.1950	30	1104.97 ± 15.87	8.70 ± 0.51
Nero d'Angeloga	Spluga	2,358	22. 8.1949	10	1051.92 ± 19.60	9.00 ± 0.71
della Malgina	Bondione	2,339	18. 9.1949	10	1068.98 ± 15.80	9.30 ± 0.30
presso Istituto Mosso	Valsesia	2,890	21. 8.1949	30	1041.88 ± 7.80	9.83 ± 0.53
al Passo S. Giacomo	Formazza	2,315	22. 9.1954	30	1124.93 ± 5.75	13.33 ± 0.65
Campo IV	Bognanco	2,293	25. 9.1951	19	1148.42 ± 6 55	15.79 ± 0.60
Campo II	Bognanco	2,225	25. 9.1951	13	1143.70 ± 12.93	16.15 ± 0.75
Cimalegna W	Valsesia	2,800	24. 8.1949	30	1167.78 ± 5.71	16.33 ± 0.45
Oriaccia	Bognanco	2,126	26. 9.1951	25	1193.89 ± 6.20	19.44 ± 0.70
Campo III	Bognanco	2,226	25. 9.1951	30	1136.16 ± 5.56	23.83 ± 0.69

TABLE 2. *Mean cephalothorax length and mean number of eggs for 14 populations of* Acanthodiaptomus denticornis *in high altitude tarns of northern Italy and Switzerland, arranged in order of increasing mean number of eggs per female*

Lake	Valley	Altitude (m)	Date	No. females	Length cephalothorax (μ)	No. eggs
di Scais	Caronno	1,449	13. 9.1949	30	1323.33 ± 13.11	5.90 ± 0.23
Fedaia	di Fassa	2,028	4.10.1949	30	953.51 ± 15.76	6.03 ± 0.46
Tenno	Varone	562	8. 7.1932	30	1161.43 ± 8.09	6.80 ± 0.27
di Molveno	di Non	821	2. 9.1931	28	1155.68 ± 5.76	9.61 ± 0.31
Monscera	Bognanco	2,071	14. 8.1947	30	1277.58 ± 5.49	16.00 ± 0.77
Pozzo del Manzo	Malenco	1,800	15. 7.1949	30	1307.18 ± 5.43	16.50 ± 0.45
Nero	Belviso	2,033	5.10.1949	10	1249.13 ± 23.85	16.70 ± 0.73
Marsch	Engadina	1,830	9. 8.1954	28	1200.88 ± 6.38	20.71 ± 0.67
della Brignola	Corsaglia	2,131	15. 8.1949	30	1324.64 ± 13.62	20.77 ± 1.06
Silvaplana	Engadina	1,794	21. 7.1950	11	1290.67 ± 9.85	21.36 ± 0.47
Sils	Engadina	1,800	20. 7.1950	30	1317.77 ± 6.25	21.47 ± 0.79
Nair	Engadina	1,880	9. 8.1954	30	1180.08 ± 4.74	26.13 ± 1.10
Campfèr	Engadina	1,794	21. 7.1950	30	1316.07 ± 7.77	31.20 ± 1.09
St. Moritz	Engadina	1,767	20. 7.1950	30	1452.01 ± 14.76	52.43 ± 0.60

TABLE 3. Arctodiaptomus bacillifer *populations arranged according to increasing mean number of eggs per female*

(1) Lake	(2) Drainage basin ($m^2 \times 10^3$)	(3) Lake surface (m^2)	(4) Ratio (2)/(3)	(5) No. eggs	(6) Length cephalothorax (μ)
1 "Ai Laghetti Lagorai	667.5	29.390	92.71	1.54	818.32
2 Gelato	550.0	85.720	6.42	1.93	998.07
3 Venerocolo	252.0	21.875	11.51	2.47	965.41
4 Coldai	372.5	20.000	18.62	2.94	990.85
5 Bleu	88.8	3.125	28.40	3.00	881.29
6 Superiore Corno d'Olen	75.0	6.000	12.50	3.20	928.51
7 Paione Superiore	554.0	13.540	40.92	3.30	941.61
8 Erdemolo	394.4	12 500	31.55	3.53	927.84
Means (Lakes 1-8)			21.58	2.74	931.49
9 Monscera	143.0	6.760	21.15	3.60	859.87
10 Bundèn	188.8	40.000	4.72	3.97	924.66
11 Paione Inferiore	961.0	13.990	68.69	4.33	1053.78
12 Nivolet Inferiore	1062.5	103.750	10.34	4.62	1077.99
13 Superiore Alpe Ligher	63.8	1.875	34.00	5.16	908.01
14 Boden S	90.6	4.000	22.65	5.79	994.54
15 Boden W	54.4	3.125	17.44	6.00	887.52
16 Antermoia	1625.0	16.900	96.15	6.17	948.47
Means (Lakes 9-16)			34.39	4.95	956 85
17 del Coston	121.9	5.000	24.37	6.18	972.87
18 della Mulattiera	32.5	625	52.00	6.32	1013.63
19 delle Valli Superiore	185.0	9.375	19 73	6.45	1164.77
20 al Rifugio Denza	157.5	7.350	21.43	7 23	1121.32
21 d'Agro	570.0	8.710	65.44	7.87	1010.93
22 Lavazza	250.0	7.500	33.33	8.23	977.99
23 Paione Medio	769.0	5.750	133.74	8.70	1104.97
24 Nero d'Angeloga	700.0	40.000	17.40	9.00	1051.92
Means (Lakes 17-24)			45.93	7.50	1052.30
25 della Malgina	1000.0	37.500	26.67	9.30	1068.98
26 presso Istituto Mosso	156.2	2.500	62.50	9.83	1041.88
27 al Passo S. Giacomo	133.8	940	142.59	13.33	1124.93
28 Campo IV	625.0	17.090	36.57	15.79	1148 42
29 Campo II	70.0	1.300	53.85	16.15	1143.70
30 Cimalegna W	312.5	7.500	41.67	16.33	1167.78
31 Oriaccia	189.0	2.850	66.31	19.44	1193.89
32 Campo III	111.9	2.190	51.08	23.83	1136.16
Means (Lakes 25-32)			60.15	15.50	1128.22

cause of loss (i.e. death) in pelagic populations has already been stated (e.g. Tonolli 1955, Bittante 1956), and of more direct concern to alpine lakes it has been found that in a group of 14 tarns (Tonolli 1954) the theoretical water renewal, based upon the volume of the lakes, the size of their drainage basins, and the intensity of precipitation, can range from a minimum of ten times to a maximum of a hundred times in a year.

Of course, these are very rough approximations because of the different degree of permeability and water capacity of the ground, and because of the possibility that inflowing water can have a temperature higher than that of the hypolimnion of the lake, so that the entire water mass of the lake is not replaced. Nevertheless, as far as the renewal of water is concerned, the average alpine lake can be placed in an intermediate position between the big lowland lake with almost standing water and the true lotic environment.

On the basis of these observations we have attempted to relate the biometrical data to the hydrographical characteristics of the lakes inhabited by the different populations. Because of the fact that the volume was unknown for some lakes, we were forced to disregard the ratio: volume of lake/area of drainage basin, and to adopt instead the ratio: area of drainage basin/area of lake. It appears evident that for the conclusions to be drawn the second ratio can also provide a good picture of the situation, since these particular species of diaptomids are much more crowded near the surface than in the deeper layers.

Table 3 contains the data of the *bacillifer* populations, with the lakes arranged according to an increasing number of eggs carried by ovigerous females. The series has then been divided into 4 groups of 8 lakes each, having the following range of numbers of eggs, respectively: 1.54–3.53, 3.60–6.17, 6.18–9.00, and 9.30–23.83. The means within the four groups of lakes agree with the hypothesis of a correlation between number of eggs and rate of water renewal. The data arranged in this way were analyzed for the "rank correlation" (Spearman 1904) between Columns 4 and 5, yielding $r_s = .565$ having a t-value of 3.750 ($<.01$ P). Hence, it is possible to argue that in our material a positive correlation exists in the foreseen way.

In order to appreciate the dispersion of the data a scatter diagram has been prepared in which the mean number of eggs in each of the 32 populations is plotted against the ratio: drainage basin/lake surface of its respective lake (Fig. 1). The top part of the same figure graphically shows the mean

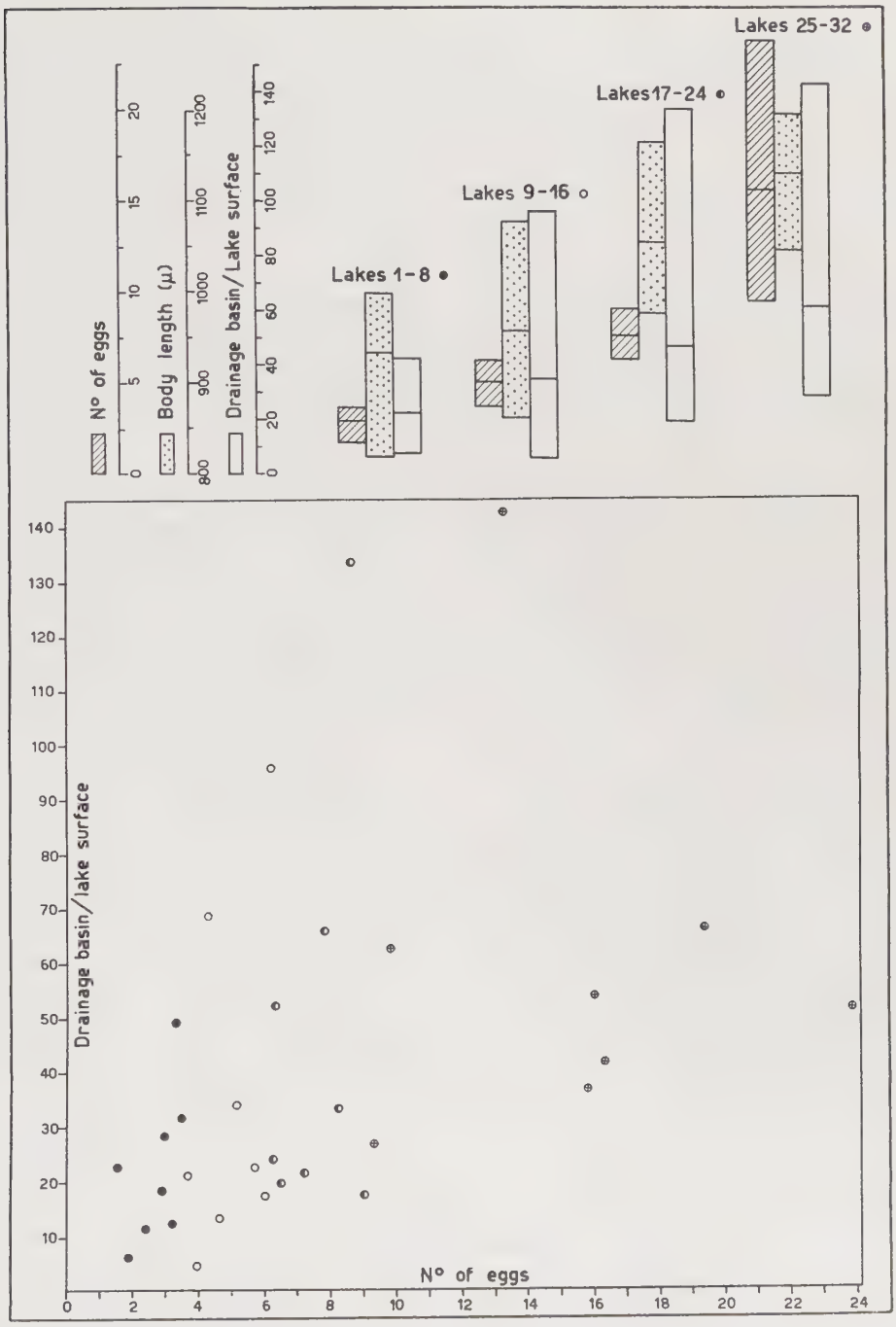

FIG. 1. *Down:* scatter diagram of mean number of eggs in different populations of *Arctodiaptomus bacillifer* against the ratio: drainage basin/lake surface of the inhabited lakes. *Up:* range and mean of cephalothorax length, number of eggs per female and of the ratio: drainage basin/lake surface for the same populations, arranged in four groups according to an increasing number of eggs. The horizontal line within each bar designates the location of the mean.

and range of variation of the three characters being related for each of the 4 groups of lakes as arranged in Table 3.

The same analytical procedure applied to the 14 populations of *denticornis* did not yield such a positive result. Four of the lakes concerned, lying at a lower altitude and having a larger volume, had already been transformed into hydroelectrical reservoirs at the time the samples were obtained. The rank correlation coefficient when calculated on the remaining 10 natural lakes, however, agrees with the result obtained for *bacillifer*.

As regards the *bacillifer* populations, the conclusion seems justified that each environment has selected larger or smaller sized females, and consequently a production of a larger or smaller number of eggs, in relation to the loss of individuals brought about in the pelagic population by the outflowing waters. This interpretation can be supported by the analogies which our problem presents in respect to the regulation of the ovodeposition in birds (Lack 1954) in relation to the fitness of the environment or to the different degree of predation pressure.

One may argue that a greater water exchange can increase the availability of nutrients and thereby bring about a larger production of eggs, on the reliable assumption that these environments have a very limited food supply. In our opinion this is not the case, because it has always been observed that the productivity in mountain lakes increases as the water exchange is reduced. Furthermore, we should have found an inverse correlation between number of eggs and altitude of lakes in relation to the different thickness of the vegetation mantle within successive altitude intervals.

The fairly good correlation found between hydrographical characteristics and ovodeposition also demonstrates that the losses by predation cannot be very high, or at least are not greatly different from lake to lake.

What has been observed for the *bacillifer* populations may possibly have a more general application. Similar phenomena might occur among most organisms dwelling in waters that have an appreciable rate of water replacement.

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The Accumulation and Exchange of Strontium by Marine Planktonic Algae[1]

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ABSTRACT

The radioactivity taken up by each of twelve species of algae, grown in a mixture of radioactive strontium and yttrium, was separated into that due to each isotope. This was accomplished by following the decay curve of the activity in the algae. The uptake of radioactive strontium and yttrium was followed at frequent intervals in *Carteria* cells grown in medium containing these two isotopes. The removal of yttrium from the medium by *Carteria* was shown to be due to adsorption on the surface, whereas strontium was taken into the cells.

It was found that *Carteria* could not divide in the absence of calcium in the medium. Neither could strontium be substituted for calcium in the metabolism of *Carteria* cells. When other factors were kept constant, the amount of strontium removed by the cells was dependent upon the strontium concentration in the medium.

With the radioisotopic dilution technique, the number of times strontium is concentrated by *Carteria* and *Nitzschia* over the amount occurring in sea water was determined. It was demonstrated that the division rate, the age of the culture, and the hydrogen ion concentration all influenced the number of times strontium was concentrated in *Carteria* cells.

Exchange experiments showed that radioactive strontium was not lost from *Carteria* cells unless the medium contained a chelating compound. Some loss of radioactive strontium from *Carteria* cells occurred when the cells were filter-washed with several different types of solutions.

INTRODUCTION

Strontium resembles calcium chemically and has been found in all phases of the biosphere in which analyses have been made (Odum 1951b). Although strontium moves in a biogeochemical cycle resembling that of calcium, it occurs in much smaller amounts in most phases. The concentration of strontium in sea water has been reported over a wide range of values and has been shown to be a conservative element varying with salinity. At 35‰ the preferred concentration for strontium is 8.10 mg/l (Odum 1951a).

The extensive distribution of strontium in the biosphere and the formation of radioactive strontium by nuclear detonations have resulted in a greater number of investigations into its role in the biotic cycle. Also the metabolism of strontium has been investigated because radioactive strontium is considered potentially one of the most hazardous of the fission products. There are several criteria by which a given fission product can be judged biotically important. Probably the three most important are its yield in the fission process, which controls the amount occurring as a pollutant in water, its half-life, and its biological absorption and retention by the organism.

With the possibility of natural bodies of water becoming polluted with radioactive strontium, it is important that the strontium cycle in aquatic environments be better understood. In the oceans the planktonic algae, because of their abundance, probably remove larger quantities of mineral elements from the water than any other form. Some elements are taken up in such large quantities that they are concentrated considerably over their amounts in sea water. Since the uptake of radioactive isotopes is usually similar to that of the nonactive isotopes of the element, it is possible that the contamination of marine waters with fission products would, in many instances, result in a rapid

[1] The work reported was carried on as a part of a cooperative project of the U. S. Fish and Wildlife Service and the U. S. Atomic Energy Commission. Publication has been approved by these Agencies.

concentration of the activity in the planktonic algae. Consequently, animals feeding upon the algae could be expected soon to incorporate into their bodies a large percentage of the radioactivity. Thus the tracing of strontium through the biotic cycle in marine waters should begin with studies of its uptake from the water by planktonic algae.

The uptake of strontium by plants and the factors controlling it have been investigated by a number of workers using radioactive strontium as a tracer. The accumulation of radioactive strontium by plants from three different types of soils has been found to be influenced by both the hydrogen ion concentration and the exchangeable calcium content of the soil (Romney *et al.* 1954). In addition to these factors, Rediske and Selders (1953), working with higher plants grown under controlled conditions of nutrient culture, observed that the strontium concentration also influenced the amount taken up by the plants. Spooner (1949) measured the concentration of strontium in several species of large marine algae. Also Knauss and Porter (1954) followed the uptake of radioactive strontium by the freshwater planktonic alga, *Chlorella*. It has been reported that in some species of plants strontium reacts much as calcium, and in *Chlorella* in particular, Walker (1953), using chemical methods of measuring strontium, observed that it could be substituted quantitatively for calcium. Even though strontium is taken up by most organisms, it is not considered an essential element for either plants or animals (Comar 1955).

Radioisotopes have been used in measuring the uptake and for elucidating the role of certain elements in the metabolism of various organisms. Investigations such as these have required the use of only one isotope at a time. However, situations may arise where it is necessary to follow the simultaneous uptake of two or more elements from the medium. Wherever pollution occurs, the possibility of more than one radioisotope occurring in the water will almost always be present. For this reason a mixture of the radioisotopes of strontium, Sr^{89} and Sr^{90}, and yttrium, Y^{90}, was used in this investigation.

The purpose of this investigation was to measure the percentage of activity due to strontium and yttrium in cells grown in the presence of these radioactive isotopes, to determine those factors influencing the uptake of strontium, to find the concentration factor for strontium in *Carteria* when grown under different conditions, and to consider the importance of algae in passing radioactive strontium into higher trophic levels.

The author wishes to thank Dr. John H. Ryther for the *Nannochloris* and *Porphyridium* cultures, and Mr. Albert W. Collier for the *Gymnodinium* culture.

MATERIALS AND METHODS

The following planktonic algae were used in this investigation:

CHLOROPHYCEAE
- *Chlamydomonas* sp.
- *Carteria* sp.
- *Thoracomonas* sp.
- *Chlorella* sp.
- *Nannochloris atomus*

CHRYSOPHYCEAE
- *Chromulina* sp.

BACILLARIEAE
- *Amphora* sp.
- *Nitzschia closterium*
- *Navicula* sp.

DINOPHYCEAE
- *Gymnodinium splendens*
- *Gyrodinium* sp.

RHODOPHYCEAE
- *Porphyridium cruentum*

Only the *Nitzschia closterium*, *Chlamydomonas*, *Carteria*, *Nannochloris* and *Porphyridium* were pure cultures. The other species were in a high state of purity, having been grown only in inorganic medium, and thus were probably sufficiently free from bacteria to make their use valid.

Radioactive strontium occurs as 12 different isotopes. The radioactive mixture which was supplied on authorization from the Isotopes Division, United States Atomic Energy Commission, and used in this investigation consisted of about 90 per cent Sr^{89} and 10 per cent Sr^{90}. The values currently listed for the half-life of Sr^{90} ranges from 19.9 years to 28.5 years, with 25 years being used most often as the average half-life. The Y^{90} which is formed through the decay of Sr^{90} has a half-life of 61 hours. After Sr^{90} has decayed for a period of time, a steady state is reached, known as secular equilibrium, where the formation of Y^{90} just equals its decay. The half-life of the mixture was 54 days when received at our laboratory.

Only an insignificant amount of any Y^{90} taken up by the cells would remain after 10 half-lives, or after about 22 days. Also the Sr^{89}, Sr^{90}, and nonactive strontium enter the cells in the same proportions as that in the culture medium, since the cells can not distinguish between these isotopes. Thus, after the Y^{90}, which entered the algae as such, decays, a state of secular equilibrium is reached in the cells. In the following experiments the activity remaining in the cells after 22 days is referred to under the collective term of "radioactive strontium". While this activity will be due to Sr^{89}, Sr^{90}, and Y^{90}, it all entered the cells as strontium and can be interpreted as radioactive strontium present at the time involved.

Samples of cells which were separated from the medium by filtering on to millipore filter paper discs were used for radioactivity measurements. These discs were then placed in plastic Petri dishes. Unless otherwise stated, the radioactivity in the cells was measured with an end-window Geiger-Müller tube connected to a scaler. Appropriate corrections were made for geometry, coincidence, background, and decay. Radioactivity measurements on all samples were made for a sufficient length of time to give a one per cent error. The thickness of the different samples never varied enough to make any significant change in the activity determination for the cells.

Natural sea water enriched with Miquel's Solutions (Rice 1953) was used in all experiments where the calcium and strontium concentrations were not controlled. When it was necessary that only certain amounts of these two elements appear in the medium, the algae were grown in an artificial sea water. This sea water is similar to that of McClendon *et al.*, described in Sverdrup, Johnson, and Fleming (1942), and that used by Ryther (1954). It was prepared by the following formula with the amounts listed in parts per thousand:

NaCl	26.5	KNO_3	0.1
$MgCl_2 \cdot 6H_2O$	2.5	$SrCl_2 \cdot 6H_2O$	
$MgSO_4 \cdot 7H_2O$	3.3	$NaSiO_3 \cdot 9H_2O$	0.023
$CaCl_2 \cdot 2H_2O$		KH_2PO_4	0.0075
KCl	0.7	$MnCl_2 \cdot 4H_2O$	0.0033
$NaHCO_3$	0.2	$ZnSO_4 \cdot 7H_2O$	0.00001

Algal cultures were grown in Erlenmeyer flasks of a capacity twice the volume of the medium used. After the appropriate number of cells and the desired amount of radioactivity had been added to the medium, the cultures were grown in a culture cabinet (Rice 1953) in constant illumination with daylight fluorescent lamps and at a temperature of 22 ± 2°C.

The number of cells per liter was determined after vigorously shaking the culture to distribute the cells evenly throughout the medium. The average of two cell counts with an improved Neubauer haemocytometer was taken as the population size. A careful check has shown that this method is very accurate for determining sizes of unialgal populations.

The water content of seven species of algae was found to be 90 per cent of the wet weight with a maximum variation of 2.2 per cent. This was obtained by first removing large quantities of cells from the medium by centrifuging for 2 to 3 hours. After the medium was decanted, the tubes were left inverted for several minutes so that water could drain from the surface of the cells packed in the bottom of them. The surface layer of cells was carefully removed and the remaining cells were placed on a very light weighing disc. Several pieces of highly absorbent paper were inserted into the mass of cells to remove any water which might have remained. They were then weighed and dried to a constant weight in a vacuum oven at 55°C. The wet weights of cells reported in these experiments were calculated on dry weight measurements.

EXPERIMENTAL RESULTS

Uptake of radioactivity by various species

Twelve species of algae were grown in the Sr^{89}, Sr^{90}, and Y^{90} mixture to measure whether or not there is a selective uptake of strontium or yttrium. Any difference in the uptake of these two radioisotopes would upset the secular equilibrium. In this parent-daughter relationship the decay rate of the parent and daughter isotopes are very different making it possible to observe selective uptake by following the decay curves of the radioactivity in the algal cells.

The algae were prepared in separate cultures with as much as 10 microcuries of the radioactive mixture per liter of medium and were grown in the culture cabinet for 10 to 12 days. Enough cells to give a rather high radioactive measurement were then filtered and the radioactivity in them determined immediately. The decay of the radioactivity in the cells was followed by periodically measuring the activity remaining in the samples for a period of 30 days, and the results are shown in Figure 1.

It was found that 10 of the 12 species investigated selectively separated Y^{90} from Sr^{90} (Fig. 1). From the decay curve of the radioactivity in *Chlamydomonas*, one of these 10 species, it can be seen that the activity in the cells on the 22nd, 25th, and 30th days was the result of strontium uptake. Since these three points represent the decay of the radioactive mixture in secular equilibrium, a straight line can be drawn through them and extended to zero time where it intersects the ordinate at a point representing 6.5 per cent. This can be interpreted to mean that, of the total activity in the *Chlamydomonas* cells at the time of the first determination, 6.5 per cent was due to radioactive strontium and 93.5 per cent was the result of Y^{90} uptake. The darkened curve, which includes the decay curves of all 10 species, indicates that most of the activity was due to Y^{90} uptake. Table 1 shows the exact per cent of radioactive strontium and Y^{90} in these 10 species of algae plus that occurring in *Carteria* and *Thoracomonas*, which were also grown and tested under similar conditions to those described above.

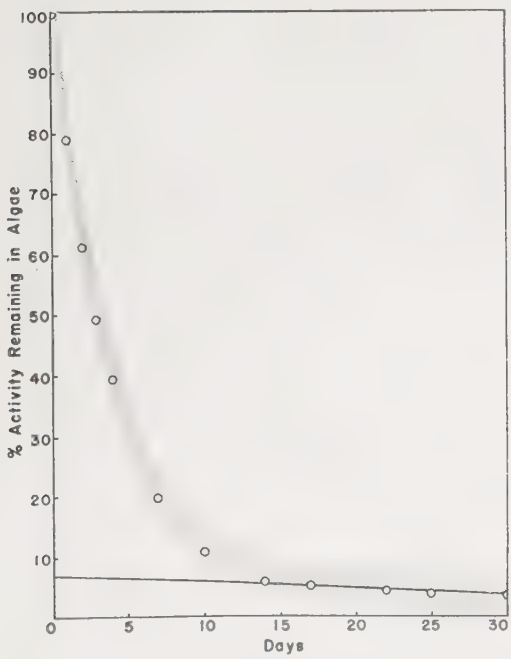

FIG. 1. The decay curves for radioactivity contained in ten of the twelve species tested fell within the darkened curve. The circles indicate the decay of radioactivity in *Chlamydomonas cells*.

TABLE 1. *Differential uptake of radioactive strontium and yttrium by algae*

Species	Percentage activity from strontium	Percentage activity from yttrium
Carteria sp.	100.0	
Thoracomonas sp.	50.4	49.6
Amphora sp.	10.0	90.0
Navicula sp.	8.5	91.5
Chromulina sp.	8.2	91.8
Chlamydomonas sp.	6.5	93.5
Nitzschia closterium	6.0	94.0
Nannochloris atomus	5.7	94.3
Chlorella sp.	5.3	94.7
Porphyridium cruentum	4.4	95.6
Gymnodinium splendens	4.1	95.9
Gyrodinium sp.	2.3	97.7

With the exception of *Carteria* and *Thoracomonas*, the other species of algae concentrated radioactive yttrium to a much greater extent than radioactive strontium. The amount of Y^{90} in the medium could not have represented much of the total activity, for only 10 per cent of the original mixture was Sr^{90} from which the Y^{90} originated. Since the Y^{90} taken up by some species represented up to 97 per cent of all the activity in them, it can immediately be seen that only a very small per cent of the radioactive strontium was removed from the medium by these cells.

Since there is such a small amount of yttrium in sea water, its adsorption on the surface of the cells would be important in its removal from the medium. The *Carteria* cells did not appear to take up much yttrium, if any, yet these cells also present a large surface area, the same as the other cells, on which the yttrium could be ad-

sorbed. The decay of radioactivity in the *Carteria* cells showed no significant deviation from the decay of the radioactive mixture, which would indicate that the radioisotopes in the *Carteria* cells after 10 to 12 days occurred in about the same proportions. If most of the Y^{90}, as well as the radioactive strontium, had been removed from the medium by the *Carteria* cells, then the decay of the activity in them could not vary significantly from that of the radioactive mixture.

Uptake of radioactivity by Carteria

Cultures of *Carteria* were prepared and the radioactive mixture was added to the medium so that the uptake of Y^{90} could be measured more carefully. These cultures were sampled periodically and the radioactivity of the separated cells was measured immediately and again after an interval of time equal to 10 half-lives of Y^{90}. From the difference in the radioactivity of the cells at the time of the initial determination and that after decay of the Y^{90}, the per cent of the radioactivity due to strontium and yttrium was calculated for the different times of exposure.

The uptake of these elements is shown in Table 2. The amount of radioactive strontium removed from the medium by the cells during the first 15 minutes is insignificant in relation to the amount in the cells after 72 hours. The uptake of Y^{90} was quite different, since more than half the Y^{90} in the cells after 72 hours was present after only 15 minutes. Even though the Y^{90} in the cells increases for each interval of time, it may not be significant, since the Y^{90} counts later in the experiment were obtained by getting the difference between two large numbers. When the Y^{90} counts in the cells are converted to represent the per cent of the total counts in them, it can be seen that, after 15 minutes, about 90 per cent is due to Y^{90}, while after 72 hours the Y^{90} accounts for only about 3 per cent.

TABLE 2. *Uptake of radioactive strontium and yttrium by* Carteria *cells*

Hours	Strontium activity (counts/min)	Yttrium activity (counts/min)	Percentage activity from yttrium
¼	69	592	89.56
6	527	735	58.24
24	4,800	848	15.01
48	23,619	898	2.47
72	34,769	948	2.65

Uptake of strontium by Carteria *as related to cell condition*

Dead cells. Cells were first killed by either heating the medium to 95°C and holding it at this temperature for 5 minutes or by adding glacial acetic acid, 1 cc per 100 cc of culture medium. After 30 minutes the cells were centrifuged from the medium containing the glacial acetic acid and resuspended in new culture medium. It was now possible to follow the uptake of radioactive strontium by cells killed in two different ways and to detect any change in the permeability of the cell membrane. The remainder of the cells, which were untreated, were retained as a control. To each of the three types of cultures 10 microcuries of the radioactive mixture were added per liter. The activity in the cells, which were sampled periodically, was not measured until after an interval of time equal to 10 half-lives of Y^{90}.

The cells in the control continued to take up radioactive strontium, while the strontium content of neither the acid treated nor the heated cells increased significantly after 15 minutes (Table 3). There are fewer counts for the acid treated than for the heated cells, since there was an unavoidable loss in centrifuging cells from the acid medium and resuspending them in the standard medium. It can be concluded from this experiment that the uptake of radioactive strontium by cells killed by these methods occurs instantaneously and is due to adsorption or exchange since it does not continue with time. Also the activity taken up by living cells during the first 15 minutes must

TABLE 3. *Comparison of radioactive strontium accumulated by living and dead cells*

Hours	Radioactivity in cells (counts/min)		
	Living	Acid killed	Heat killed
¼	64	38	70
24	5,658	37	56
48	11,814	36	69

TABLE 4. *Comparison of uptake of radioactive strontium by non-dividing and dividing cells*

Hours	Dividing		Non-dividing	
	Population (cells × 10^7)	Radio-activity (counts/min)	Population (cells × 10^7)	Radio-activity (counts/min)
¼	6.3	67	6.3	54
6		846		66
24	20	6,226	6.3	180
48	52	34,744	6.3	118
72	91	75,830	6.3	82

be due to adsorption or exchange, since it was not significantly larger than that removed by the dead cells.

Dividing cells. The amount of strontium removed from the medium due to growth can be measured by comparing the amount of radioactive strontium taken up by a dividing cell with that of a non-dividing cell. A culture of *Carteria* cells was placed in the dark for 7 days during which time cell division ceased. Two new cultures were started from this culture with 9 microcuries of the radioactive mixture per liter. One was retained in the dark and the other was placed in the culture cabinet in the light. The cell population size increased rapidly in the culture placed in the light, while there was no detectable change in the population size in the culture kept in dark (Table 4). The radioactive strontium continued to enter the cells in the light, while there was no further increase in uptake after 24 hours by the cells kept in the dark. The amount of radioactive strontium taken up as a result of adsorption and/or exchange by each cell in the light, if related to time, could not be greater than that taken up by a cell in the dark. The average amount of radioactive strontium taken up *per cell* in the light was 64 times as much as that taken up by a cell in the dark. It was shown in the control of the previous experiment that the amount of radioactive strontium taken up by cells increases as the population size increases. It has now been demonstrated in this experiment that dividing cells remove much more radioactive strontium *per cell* from the medium than non-dividing cells. Thus the greatest uptake of strontium must be due to or related with absorption and the formation of new protoplasm.

TABLE 5. *Effect of division rate and age of culture on accumulation of radioactive strontium*

Day	Rate (divisions/day)		Strontium (counts/min/10^4 cells)	
	Culture 1	Culture 2	Culture 1	Culture 2
1	1.59	1.00	6.2	3.4
2	1.30	0.30	13.8	8.2
3	0.75	0.57	16.6	9.0

Cells dividing at different rates. The question now is whether there is any difference in the amount of radioactive strontium *per cell* when the cells are dividing at different rates and when the cultures have grown for different periods of time. Culture 1 was started with 6.3 × 10^7 cells per liter from a culture which had been kept in the dark prior to this experiment. Culture 2, prepared with cells grown in the light in medium containing no phosphorus, was started with 19 × 10^7 cells per liter. Both cultures were started with 9 microcuries of the radioactive mixture per liter.

Cells in Culture 1 had a faster rate of division than Culture 2 (Table 5). Also the amount of radioactive strontium in each cell in Culture 1 at the end of each interval of time was larger than that occurring per cell in Culture 2. From the data presented in Table 5 it appears that the faster the rate of division, the faster radioactive strontium enters a cell. It is of further interest to note that, regardless of the rate of division, more radioactive strontium occurs in a cell the older the culture at the time of sampling. At least this holds for the intervals of time tested in this experiment.

Uptake of calcium and strontium by Carteria *in relation to their concentrations in the medium*

To find the effect of the calcium concentration on the uptake of strontium and vice versa, artificial sea water was devised in which the calcium and strontium concentrations could be varied. The growth of *Carteria*, as well as that of several other species, was as good as, or better than, that obtained in culture medium prepared by adding nutrients to natural sea water.

Effect of calcium concentration on uptake of

strontium. Whether or not the calcium concentration has any effect on the uptake of strontium was followed in *Carteria* cultures which were prepared with the same size inoculation of cells but with different concentrations of added calcium (Table 6). The strontium concentration was the same in all cultures, and 5 microcuries of the radioactive mixture were added per liter. Thus the uptake of a given per cent of radioactive strontium in any culture represented the removal of the same total amount of strontium. In Table 6 it can be seen that about the same per cent of radioactive strontium was removed from the culture medium with the three highest concentrations of calcium. The cells in the cultures with 0.075 grams of calcium per liter removed a little more strontium, while the cells in cultures with no added calcium took up the largest per cent of strontium. It should be pointed out that the number of cells obtained in all cultures with added calcium did not vary significantly from 56×10^7 cells per liter, while cultures with no added calcium reached a population of only 8×10^7 cells per liter. Since without added calcium in the medium the cells could not divide even in the presence of excess strontium, it can be concluded that strontium cannot be substituted for calcium.

In the cultures with no added calcium there were fewer cells and somewhat more uptake of strontium; thus the amount of strontium *per cell* was considerably more. Since calcium is closely related with the permeability of the cell membrane, the added uptake of strontium in the cultures with the lower calcium concentrations may be the result of a change in permeability of the membrane.

Effect of strontium concentration on uptake of calcium. The effect of strontium on the uptake of calcium by *Carteria* cells was

TABLE 6. *Effect of calcium concentration on uptake of strontium*

Calcium added (g/l)	Percentage radioactive strontium in cells
0.300	40.0
0.275	38.6
0.150	38.9
0.075	45.6
0.000	54.1

TABLE 7. *Effect of strontium concentration on uptake of calcium*

Strontium added (g/l)	Percentage radioactive calcium in cells
0.011	1.5
0.008	1.3
0.005	1.4
0.003	1.1
0.000	1.3

tested by starting cultures with the same size inoculation of cells in artificial sea water containing different concentrations of added strontium (Table 7). The calcium concentration was the same in all cultures, and 9 microcuries of Ca^{45} were added per liter. In Table 7 it is shown that about the same per cent of Ca^{45}, and therefore total calcium, was removed from all cultures, regardless of the strontium concentration. It can be concluded that the uptake of calcium by *Carteria* cells is not influenced by these concentrations of strontium.

The population size reached in the cultures to which no strontium was added was 50×10^7 cells per liter, while in all cultures to which strontium was added it did not vary significantly from 60×10^7 cells per liter. It was shown in the previous experiment that cells could not divide without added calcium Here it can be seen that cells divide almost as fast in the absence of as in the presence of *added* strontium. It appears that strontium is not very necessary for the growth of *Carteria*, or if needed, then small amounts of strontium entering the artificial sea water as impurities are sufficient to meet the strontium requirements, or, finally, that calcium can be substituted for strontium.

Effect of strontium concentration on uptake of strontium. Carteria cells were grown in three different concentrations of strontium in order to determine the effect of the concentration in the medium on the uptake by the cells. Three artificial sea water media were prepared: one with 11 milligrams of strontium and 5.4 microcuries of the radioactive mixture per liter, one with 55 milligrams of strontium and 27 microcuries per liter, and one with 110 milligrams of strontium and 54 microcuries per liter. Cultures for each concentration were inoculated with

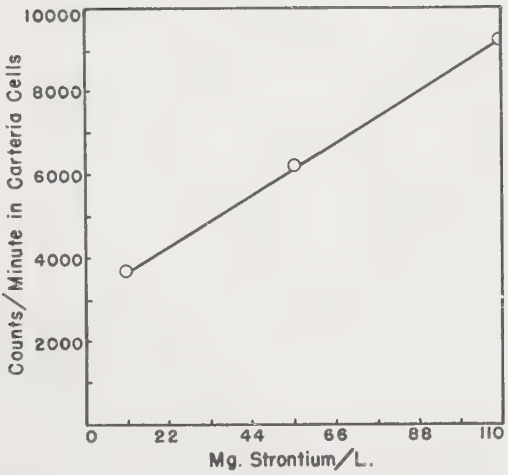

FIG. 2. Uptake of strontium in relation to its concentration in the medium.

the same number of cells. After eight days in the culture cabinet in the light, the cells were filtered from the medium and the radioactivity in them was measured. The specific activity was the same in each culture; therefore, the uptake by the cells of a given amount of activity in any culture represented the uptake of the same amount of total strontium.

The uptake of strontium by the cells increased as the concentration of strontium in the medium increased, and the amounts of strontium taken up at these three concentrations formed a straight line when graphed (Fig. 2). A five fold increase of strontium in the medium caused, roughly, a doubling of the amount of strontium in the cells, while 10 times as much strontium in the medium resulted in a tripling of the quantity of strontium in the cells. The number of cells per liter at all three concentrations did not vary significantly from each other. It can also be concluded from these observations that concentrations of strontium as high as these exert no toxic effects upon the division rate of *Carteria*.

Conditions affecting the concentration of strontium in Carteria *cells*

The concentration of an element by a marine organism can be determined more easily, in most instances, with radioisotopes than by ordinary chemical analysis. The radioisotope of the element to be studied is first added to a known volume of sea water. The radioactivity per aliquot volume of water is then measured and the test organism is placed in the water. The organism must remain in the active sea water until no more activity enters it and until a state of equilibrium is reached between the activity in the water and that in the organism. At this time the isotope will have been distributed at random wherever the element occurs. After a state of equilibrium has been reached, as determined by measuring the activity in aliquot samples of water removed periodically, the activity remaining in the water is compared with that originally present. The volume of sea water which would have been necessary to add to the original volume to reduce the activity in an aliquot portion to the same level as that resulting from the addition of the organism is now computed. The wet weight of the organism is then divided into the weight of this volume of water to determine the number of times the organism has concentrated the element. Since there is a radioactive parent element producing a radioactive daughter, the extent to which one element is concentrated is more difficult to determine than when only one radioisotope is used, but can be ascertained by following the decay curve. The method used was developed by Spooner (1949) for measuring the concentration of strontium by large algae.

Rate of division. In a previous experiment it was found that more strontium was taken up *per cell* when the rate of division was faster. It was now of interest to determine whether faster dividing cells will also concentrate more strontium per unit weight. Two identical cultures of *Carteria* were prepared with 12.3 microcuries of the radioactive mixture in each culture. One culture was placed in the culture cabinet in the light and the other was grown in natural daylight. The cells grown in natural daylight divided at a much slower rate with 5.18 grams of cells being harvested after 24 days, while there were 5.20 grams of cells after 13 days in the culture grown in the culture cabinet. After these intervals of time samples of medium from which the

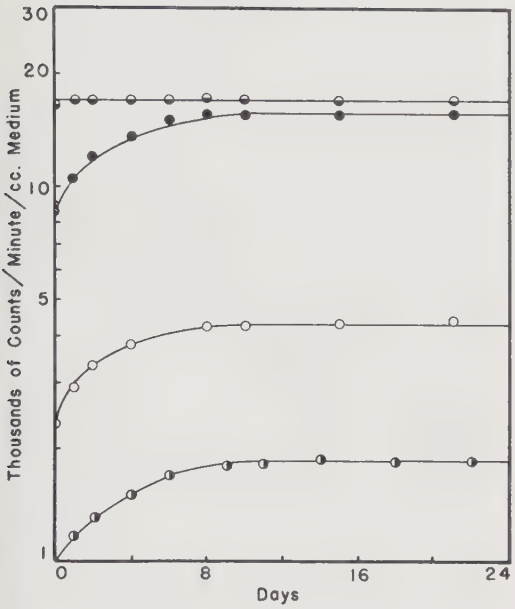

FIG. 3. Decay curves for medium in which *Carteria* and *Nitzschia* were grown.

◒ Control medium in which no cells were grown
● Medium in which *Nitzschia* was grown in culture cabinet
○ Medium in which *Carteria* was grown in natural daylight
◑ Medium in which *Carteria* was grown in the culture cabinet

cells had been removed by filtering were dried and measured for their radioactivity. A control had been prepared at the beginning of the experiment by adding 12.3 microcuries of the radioactive mixture to a liter of culture medium containing no *Carteria*. Radioactivity in samples of medium from the *Carteria* cultures were counted periodically and compared with samples prepared in a similar manner from the control and counted over the same interval of time. Cultures of *Nitzschia* had also been prepared with 12.3 microcuries of the radioactive mixture per liter and grown in the culture cabinet and in natural daylight. Preparation and counting of radioactivity in the medium was similar to that used for the control and for *Carteria*. The decay curves for the control, for the *Carteria* cultures grown in the cabinet and in the window, and for the *Nitzschia* grown in the culture cabinet are shown in Figure 3. The radioactive mixture had been in our laboratory for some time prior to this experiment, and most of the Sr^{89} had decayed, as revealed by the insignificant drop in the number of counts per minute in the control samples over the interval of 22 days during which counts were made. This longer half-life indicated most of the radioactive strontium present was Sr^{90}.

The decay curves for the medium which contained the algae show an increase in radioactivity for about 8 to 10 days. This was due to more Y^{90} being formed by the decay of Sr^{90} than was decaying. After secular equilibrium was reached in the *Carteria* and *Nitzschia* culture media, the decay curves level off. From this time on the rate of decay is similar to that of the control. The per cent of the original activity remaining in the *Carteria* and *Nitzschia* culture media at this time was calculated and the concentration factors computed. The formula for determining the concentration factor is as follows:

$$\frac{\text{Weight of medium} \times \text{Per cent activity in cells}}{\text{Wet weight of cells} \times \text{Per cent activity in medium}} = \text{Concentration factor}$$

It was found that *Carteria* cells concentrated strontium to a greater extent when grown in the culture cabinet where cell division was faster. The concentration factor for cells grown in the cabinet was 1,616 while it was only 561 for those grown in natural daylight. Also, regardless of whether *Carteria* cells were grown in the cabinet or in natural daylight, they concentrated much more strontium than did *Nitzschia* cells. *Nitzschia* cells concentrated strontium only 17 times over the concentration occurring in sea water when grown either in the culture cabinet or in natural daylight.

Age of culture. The concentration factor for *Carteria* cells was followed in the same cultures over a period of several days. Since adsorption and exchange occur rather rapidly and the *Carteria* cells divide about once a day for the first few days, it was believed that within three days the radioactive strontium would be distributed

TABLE 8. *Increase of strontium concentration in* Carteria *with age of culture*

Age in days	Concentration factor
3	100
6	243
10	337
18	1,244

at random wherever strontium occurred. Thus the first determinations were made on the third day after the culture was started and again on the 6th, 10th, and 18th days. The methods used in this experiment are the same as those discussed in the previous one. The cells continued to increase in weight with time. Also, the per cent of radioactive strontium in the cells and the concentration factor for strontium continued to increase with time (Table 8).

Hydrogen ion concentration. The biological availability to algae of many elements for uptake and utilization in metabolic processes depends upon the element existing in an ionic state in the water. Since the ionic state of many elements is closely related to the hydrogen ion concentration, the availability of strontium to *Carteria* grown in culture medium with the hydrogen ion concentration initially adjusted to pH 6.0 and pH 9.0 was determined. The control consisted of a culture started with the regular culture medium which had a pH of 8.2. Hydrochloric acid was used to reduce the medium to pH 6.0 and sodium hydroxide was added to raise the medium to pH 9.0. The cultures contained 12.3 microcuries of the radioactive mixture.

Carteria cells grown in culture medium with pH 8.2 concentrated strontium 975 times over its amount in sea water. The concentration factor for cells grown in medium with an initial pH of 6.0 was only 223, while that in medium with a pH 9.0 was even less, being only 85. The cells in the culture with a pH 6.0 were larger and weighed more than those in the control culture, even though the cells in the control culture divided a greater number of times. All cultures were started with the same number of cells, but counts just prior to weighing showed the largest population in the control culture. Again the highest concentration factor occurs where there has been the greatest number of cell divisions. The lower concentration factors for cells grown at pH 6.0 and pH 9.0 may be due not only to the strontium being less available for uptake at these hydrogen ion concentrations, but may also be partially due to the reduced rate of division.

Loss of radioactive strontium from Carteria *cells*

Whether an element remains in an exchangeable state once inside a cell depends upon the kind of compounds into which it is bound. If an element is loosely bound or bound into compounds with a rapid rate of turnover, then the possibility of exchange is greater. Also elements which remain in the cell in what has been referred to as the "metabolic pool" may be exchanged more easily than other elements bound in tissue.

Carteria cells which had grown in the presence of the radioactive mixture for 12 days were fractionated to determine the distribution of strontium. It was found that more than 97 per cent of the activity contained in the cells was soluble in TCA (trichloracetic acid). The fractionating procedure was similar to that used by Rice (1953). Also *Carteria* cells were fractionated in alcohol and in ether. Less than 0.1 per cent of the activity was lost from the cells to either solution.

Resuspended in non-radioactive culture medium. The loss of strontium from *Carteria* cells was followed by centrifuging cells from a 5-day-old culture which had been started in artificial sea water with 10 microcuries of the radioactive mixture per liter, and resuspending them in another portion of artificial sea water containing no radioactive strontium. This culture was then divided into two cultures and to one enough strontium was added to give the same concentration as occurs in natural sea water. The cultures were returned to the culture cabinet and the medium was sampled after 1, 5, 24, and 48 hours. The radioactivity was measured in both the cells and medium under comparable conditions. The cells

from 25 cc of medium at zero time contained 6651 counts per minute. From these data it can be computed that less than 2 per cent of the radioactivity in the cells was lost to the medium, even after 48 hours.

Resuspended in culture medium containing a chelating substance. The radioactive strontium contained in the cells that were resuspended in nonactive medium in the previous experiment may have moved back and forth between the cells and medium, even though it could not be detected in the medium. Thus the experiment was repeated with a chelating compound, Versene, a sodium salt of ethylene-diamine-tetraacetic acid, added to the medium. This compound can bind elements leaving cells into such large molecules that they can not reenter the algae. The use of chelating compounds has been extensively studied by Hutner, Provasoli, Schatz, and Haskins (1950). With chelating agents metal ions can be reduced in cultures to levels that are not harmful, or deficiencies of some elements may be created so complete as to reduce the division rate.

A *Carteria* culture was prepared in artificial sea water with 10 microcuries of the radioactive mixture per liter. After growing in this medium for 7 days, the cells were centrifuged and resuspended in another volume of artificial sea water containing neither active nor nonactive strontium. This culture was divided into two parts and different amounts of the chelating agent were added to each (Table 9). The cells were filtered out of the medium, and the radioactivity in the medium was counted with a dip-type Geiger-Müller counting tube at frequent intervals of time over a 24-hour period (Table 9). It was found that radioactive strontium did migrate out of the cells, the amount in the medium increasing with time for the first two hours. A greater amount of radioactive strontium, and therefore total strontium, was found in the medium containing the larger quantity of Versene.

TABLE 9. *Loss of radioactive strontium from* Carteria *cells resuspended in nonactive medium containing a chelating substance*

Cells from sample volume initially contained 8391 counts per minute.

Hours	Radioactivity in filtered medium (counts/min)	
	With 0.5 g Versene/l	With 1.0 g Versene/l
½	764	1694
1	1223	2646
2	1654	3226
4	1550	3408
7	1675	3425
24	1525	3250

TABLE 10. *Loss of radioactive strontium from living and dead* Carteria *cells when filter washed with different types of solutions*

Washing solution	Percentage loss	
	Living cells	Dead cells
Culture medium		15.49
Hot culture medium		24.77
Culture medium (with 10X Sr in sea water)	2.09	
Culture medium (pH 5.4)	7.73	
Culture medium (pH 3.0)	29.52	
Culture medium (pH 2.0)	37.85	
Distilled water	7.19	13.65
Hot distilled water	24.20	24.10

In filter-washing. The instantaneous loss of strontium from both living and dead cells can be conveniently measured by a filter-washing technique. In these experiments the *Carteria* cells, after growing 8 to 12 days in medium containing up to 10 microcuries of the radioactive mixture per liter, were filtered on millipore filter discs and washed with different types of medium. The radioactive strontium remaining in the cells after washing was compared with that present before washing to determine the per cent lost. Cells, first killed by drying at 70°C for 4 hours after having been suspended on the filter, were also washed to determine whether more strontium would be exchanged or lost from dead cells such as these or from living cells, since the permeability of the cell membrane may be different in dead cells.

There was not much difference in the amount of radioactive strontium lost from living and dead cells washed with distilled water (Table 10). In another experiment it was found that only 35.4 per cent of the radioactive strontium was lost from living *Carteria* cells suspended in distilled water for as long as 65 hours. The per cent of

radioactive strontium lost from living *Carteria* cells was large when filter-washed with culture medium having higher hydrogen ion concentrations than those occurring in sea water. It can be stated that most of the radioactive strontium contained in living cells is retained inside the cell, even when subjected to unfavorable media in filter-washing. Also, cells that have been dead for only a short period of time do not lose large quantities of radioactive strontium when filter-washed.

DISCUSSION

In this investigation the algal cells were grown in medium containing Sr^{89}, Sr^{90}, and Y^{90}. Ten of the twelve species tested became more radioactive from the uptake of Y^{90}, which was present in very small amounts. In the other two, the quantity of Sr^{89} taken up masked the uptake of the yttrium. This differential uptake was easily followed by observing the decay characteristics of the radioactivity of the cells. These observations emphasize that care must be taken in considering the radioactivity of biological materials when more than one radioactive element is used, even though one or more of the radioisotopes are present in trace amounts.

It should also be mentioned that upon addition of the radioactive mixture to sea water some yttrium occurs as a radiocolloid. Some of this colloidal material can be filtered and centrifuged from the culture medium. However, when the radioactive material is added to cultures of algae the amount obtained by either filtering or centrifuging the cells from the medium is several times that obtained in the absence of the cells. Since there is such a small amount of yttrium in sea water and since its removal by the cells is instantaneous, it is probably correct to assume that almost all of the yttrium is taken up by the algae.

The surface area of algal cells plays a very important role in removal of some nutrients from culture medium. In most investigations the uptake of an element is usually reported without much consideration of the amount adsorbed on the surface. For many elements adsorption on the surface may be only the precursor to its entry into the cell, but for other elements that cannot penetrate the cell membrane, or do so slowly, it may be a "retention area". The adsorption of an element onto the surface of a cell may be most important for those elements occurring in the medium in relatively small amounts or for those elements taken up by the cell in small quantities. If only a small trace of the element occurs in the medium, then the large surface area available on the cells may result in most of the element being adsorbed on the cell, regardless of whether or not it serves as a nutrient. On the other hand, if not much of an element is utilized in metabolism, the adsorption of small amounts on the surface may represent a significant portion of that taken up by the cell. In this last instance the actual requirement of the cell for the nutrient would be considerably less than the amount removed from the medium.

It was found that the adsorption of yttrium was significant in its total uptake, while the surface area retained an insignificant portion of strontium when compared to that entering the cells. The uptake of yttrium was almost complete within 15 minutes after adding the radioactivity. As far as could be ascertained no data have been published showing the need for yttrium in the metabolism of algae. While some of the Y^{90} uptake may have been due to exchange, Spooner (1949) believes uptake as a result of adsorption in higher marine algae greatly exceeds that taken up through exchange. There are numerous other elements occurring in sea water in trace amounts which could also be adsorbed in considerable amounts just as readily as yttrium. The importance of adsorption on the surface of cells should be investigated further, since this may be the only explanation in some instances for the occurrence of some trace elements in algae. Stiles (1951) has stated that analysis has shown higher plants to contain small amounts of many elements not generally regarded as essential.

Very little strontium was taken up during the first 15 minutes in these experiments. Living cells, during the first 15 minutes, were found to take up no more

activity than dead cells. Also living but non-dividing cells after 72 hours contained only one sixty-fourth the amount of strontium *per cell* as in dividing cells. If all the uptake by the non-dividing cells was due to adsorption, it can be seen that it still would be a very small per cent of that taken up by dividing cells. It seems that surface adsorption of strontium is of very minor importance in considering the uptake of this element.

The possibility of the *division rate* influencing the amount of an element in a cell has been considered in this investigation. It was found that during the first 72 hours more strontium entered a cell the faster the rate of division. Also it was shown that the concentration factor for strontium was larger for cells grown in the culture cabinet where cell division was more rapid than for cells grown in natural daylight. Krauss (1953), in measuring the nitrogen accumulation by *Scenedesmus* in medium with and without the three micronutrients, boron, zinc, and manganese, found that the uptake of nitrogen proceeded almost as rapidly in the cells where growth was limited by micronutrient deficiency as in cells growing at a normal rate. This resulted in the uptake of more nitrogen per cell in the culture medium with a nicronutrient deficiency or in the culture with the slower rate of division. That the rate of division may have no effect on the amount of an element *per cell* was observed in the uptake of cesium by *Nannochloris* cells (Rice, unpublished). In this experiment the uptake curve for cesium was parallel to the growth curve, indicating the uptake of an equal amount of cesium with the formation of a given quantity of cells throughout the entire growth phase.

From the data presented above it can be concluded that some elements enter cells in larger amounts when the division rate is faster, while other elements occur in larger amounts in more slowly dividing cells. Finally, there are some elements that enter cells at the same rate regardless of the division rate. It is usually difficult to change the rate of division without altering the chemical composition of the medium, so that the different rates of uptake mentioned as related to division rate could possibly be due to other factors. It is believed that this problem should be investigated further, and it is suggested that it could be accomplished either by starting cultures identically, except for the initial inoculation of cells, or by changing the intensity of light. It has already been shown many times that both the rate of division and total population size reached in algal cultures can be influenced by the number of cells used to start new cultures.

Walker (1953) showed that the calcium requirement in *Chlorella pyrenidosa* is almost quantitatively replaced by strontium. In a later investigation Walker (1954) found that closely related members of the Chlorophyceae differ widely in their ability to replace calcium with strontium. The experiments conducted in this investigation revealed that strontium could not be substituted for calcium in *Carteria*. It was further observed that calcium is essential for cell division and must be present in the medium before any significant amount of division occurs.

The calcium requirement for a marine diatom has been established at approximately 0.5 mg/100 cc culture medium (Hutner 1948). In the present investigation the effect of the strontium concentration upon calcium uptake by *Carteria* was measured with radioactive calcium. It was found that approximately the same per cent of total calcium was removed from the medium by *Carteria* cells. Since the artificial sea water was prepared with 0.3 grams of calcium per liter, the cells removed from 3.3 to 4.5 mg of calcium per liter. The highest of these amounts removed occurred in artificial sea water containing approximately the same concentration of strontium as sea water.

One of the most important factors affecting the amount of an element accumulated by planktonic algae is the concentration of the element in the culture medium. Ketchum (1939) has shown that the amount of phosphorus entering *Nitzschia closterium*, a marine diatom, depends upon the concentration of phosphorus in the medium, other

factors being kept constant. The uptake of cesium by *Nannochloris*, a planktonic alga, was found to be proportional to the cesium concentration in the medium (Rice, unpublished). Also the accumulation of strontium in both higher plants (Rediske and Selders 1953) and in *Chlorella pyrenoidosa* (Knauss and Porter 1954) was found to be proportional to the concentration in the nutrient medium. The amount of strontium was also found to increase in *Carteria* cells as the concentration in the medium was increased. However, with a five-fold increase of strontium in the medium there was roughly a doubling of the amount of strontium entering the cells, and with ten times as much strontium in the medium there was approximately three times as much strontium taken up by the cells. The concentration of strontium tested with *Chlorella* was only over a range from 1.0 to 40 milligrams per liter, while in the present investigation the highest concentration used was 110 milligrams per liter. The smaller increase of strontium in *Carteria* cells than that observed in *Chlorella*, with an equal increase of strontium in the medium, was probably due to one or both of the following conditions: (1) the uptake of strontium by *Carteria* cells was less because two of the three concentrations tested were higher than those used by Knauss and Porter, and/or (2) the difference in the total quantity of nutrients and in their ratio one to another in the artificial sea water was such that less strontium was taken into the *Carteria* cells.

The amount of strontium found in plants appears to be related to the age of the plant. Rediske and Selders (1953) found the greatest concentration of strontium always in the oldest leaves, with a progressively smaller concentration as the age of the leaf decreased. While the age of leaves of higher plants may have no physiological property analogous to the age of a microorganism culture, in the present investigation it was observed that, regardless of the division rate, the amount of strontium per cell increased with the age of the culture for the time intervals tested. Also the concentration factor, which was determined after 3, 6, 10, and 18 days in one set of experiments, showed that more strontium occurred in a gram of cells after each interval of time. From these observations it appears that strontium continues to enter the cells with time, and that the amount entering the cells is not entirely related to the formation of new tissue. A possible explanation for this continuous uptake may be that the cells cannot prevent the strontium from entering and are not able to eliminate it, so that the amount per cell or per gram continues to increase with time.

The hydrogen ion concentration was found to control not only the rate of division of *Carteria* cells but also the amount of strontium concentrated by the cells. Whether the concentration of strontium was biologically unavailable due to the formation of certain strontium compounds or less strontium was removed because of the slower division rate of *Carteria* in the cultures with pH 6.0 and 9.0 is not known. The population in the culture started at a pH of 6.0 weighed more but had fewer cells than that in the culture started at pH 8.2.

It was found that no significant amount of radioactive strontium accumulated in the culture medium in which cells containing radioactive strontium were suspended. The results were the same whether or not the medium contained strontium. With the addition of a chelating agent the loss of radioactive strontium from the cells could be demonstrated. The order of chelation at a pH 8.2 is calcium, magnesium, and strontium. Also, it can be computed theoretically that it would require about 31.5 grams of Versene per liter to chelate any strontium leaving the *Carteria* cells. Since only two concentrations of Versene, 0.5 and 1.0 gram per liter, were used in the experiment reported here, it may seem improbable that chelation was responsible for the results observed. Nevertheless, radioactive strontium did leave the cells and accumulate in medium containing Versene. If not due to the phenomena of chelation it was brought about by some other change associated with the presence of the Versene. In this experiment the medium contained

no strontium, so that this is not a demonstration of strontium exchange. Rather, it was only a short-circuiting of the strontium cycle between medium and cells, showing that strontium can leave the cells as well as enter them.

It was further shown that strontium is lost from *Carteria* cells by filter-washing. In this type of experiment media or other solutions used to wash the cells remain in contact with them for only a short interval of time. Appreciable quantities of radioactive strontium were lost from living cells to only three washing solutions: hot distilled water, culture medium with a pH of 2, and one with a pH of 3. Thus it appears that even though strontium may not be very important in the metabolism of the cells, it is not readily lost from them.

Some species of planktonic algae may not be very instrumental in concentrating and passing strontium into higher trophic levels. Of the twelve species tested, *Carteria* and *Thoracomonas* were the only forms that concentrated strontium to any great extent. The yield of cells in grams per liter is no doubt higher in laboratory cultures than ordinarily found in nature. A filter-feeding animal in sea water with a population of *Nitzschia closterium* as large as those obtained in these experiments could still obtain just over 3 grams of cells from a liter of water. Since the *Nitzschia* cells concentrated strontium only 17 times over its concentration in sea water, an animal filtering all the cells from a liter of water and incorporating all the strontium from the cells into its own body would only have a total amount of strontium equivalent to that occurring in about 51 cc of sea water. However, animals feeding upon *Carteria* cells would have a much larger supply of strontium from an equivalent weight of cells since *Carteria* was found to concentrate strontium up to 1616 times over the concentration in sea water. Other factors should be taken into consideration in discussing the role of phytoplankton as a source of strontium for animals, including the relatively high concentration of strontium in sea water and the large volume of water filtered by many filter-feeding animals. If these organisms can remove strontium from the sea water to meet any metabolic requirements they may have, it would appear that the added strontium from the phytoplankton food may not be a significant contribution.

SUMMARY

1. All twelve species of planktonic algae grown in medium containing Sr^{89}, Sr^{90} became radioactive. Only in *Carteria* and *Thoracomonas* was this radioactivity due chiefly to the uptake of radioactive strontium. The greatest part of the radioactivity in the cells of the other species was due to Y^{90}, the radioactive daughter of the Sr^{90}.

2. In *Carteria* there was almost instantaneous uptake of Y^{90}, which did not increase greatly with time and was therefore apparently the result of adsorption of Y^{90} on the cell surfaces. The radioactivity resulting from the uptake of radioactive strontium was of little significance at first, but gradually it became the chief source of the radioactivity.

3. Dead and living, but non-dividing, cells did not remove any significant quantities of strontium from the medium. With dividing cells there was continued uptake; the faster the rate of division, the greater the amount of strontium taken up per cell during the first 72 hours after starting the culture.

4. Calcium at higher concentrations did not influence the amount of strontium taken up by *Carteria* cells. Also calcium was found to be essential for cell division. It was further found that strontium could not be substituted for calcium.

5. The strontium concentration did not influence the amount of calcium taken up by *Carteria* cells. When other factors were kept constant the amount of strontium removed by the cells was dependent upon the strontium concentration in the medium.

6. It was found that *Nitzschia closterium* concentrated strontium 17 times over its concentration in sea water. The concentration factor for strontium in *Carteria* was found to vary, being influenced by the rate of cell division, the age of the culture, and the initial pH of the medium in which the

cells were grown. However, it was much greater than that for *Nitzschia*.

7. Little radioactive strontium was lost or exchanged from *Carteria* cells when resuspended in non-radioactive medium. The addition of a chelating substance to the medium demonstrated that strontium does leave the cells. Also, very little radioactive strontium was lost from radioactive *Carteria* cells suspended on a filter and washed with various washing solutions.

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